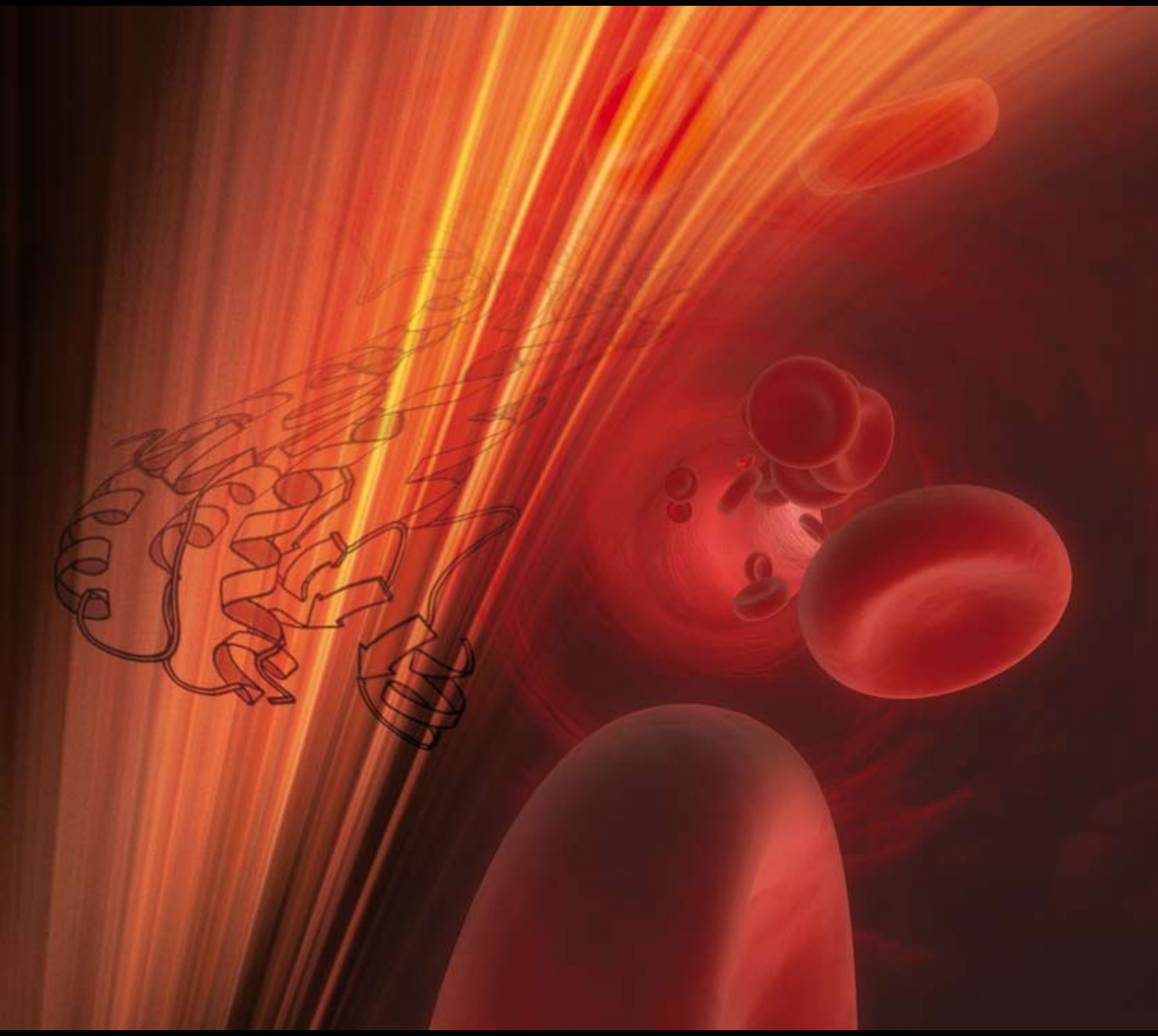
PPAR Research

PPARs and RXRs in Male and Female Fertility and Reproduction

Guest Editor: Pascal Froment



PPARs and RXRs in Male and Female Fertility and Reproduction

PPAR Research

PPARs and RXRs in Male and Female Fertility and Reproduction

Guest Editor: Pascal Froment

Contents

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 637490, 2 pages
doi:10.1155/2008/637490

Editorial

PPARs and RXRs in Male and Female Fertility and Reproduction

P. Froment

Unité de Physiologie de la Reproduction et des Comportements, UMR 6175 INRA-CNRS, Université F. Rabelais de Tours Haras Nationaux, 37380 Nouzilly, France

Correspondence should be addressed to P. Froment, pfroment@tours.inra.fr

Received 9 December 2007; Accepted 9 December 2007

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors controlling many important physiological processes, including energy homeostasis, lipid, and glucose metabolism, inflammation, as well as cell proliferation and differentiation. PPARs and their heterodimeric partner (retinoid X receptor, RXR) have been found in early embryo, developing fetus, and in various compartments of the reproductive system (hypothalamus, pituitary gland, ovary, uterus, and testis) of many species (birds, fishes, mammals such as rodents, cattle, pigs, and humans) [1]. The reviews in this special issue paint a broad picture of the potential role of the PPAR-RXR system in the reproductive axis. They also raise new questions about the biological actions of the PPAR-RXR system in reproduction and its possible manipulation in treatments of fertility disorders (due to problems with energy metabolism, or specific diseases).

Over the last 10 years, a number of studies in vivo and in vitro have strongly suggested that these nuclear receptors might play an important role from gametogenesis to parturition, including gestation and the links mother/fetus [2]. Thus, PPARs are expressed in the testis where lipid metabolism and specially the β-oxidation of fatty acids are important for testicular functions (steroids synthesis, lipid composition of the sperm, etc.). In addition, some testicular toxicants such as phthalates bind to PPARα and PPARγ and modify their activities. In female, invalidation of PPARγ in the mouse ovary [3] leads to a decrease in fecundity probably due to a drop in the production of sexual steroids. Mice null for PPARβ/δ, PPARγ, or RXRα [4–6] display alterations in the attachment of embryos to the endometrium and/or placenta development and function. During the labor, mRNA expression of cyclooxygenase-2, an inducer of contractions of the myometrium and a PPAR target gene, is increased in fetal membranes when the PPARα and γ expression drops at the start of parturition. After birth, PPARs continue to play a role in the relation mother/neonates through the mammary gland functions. Indeed, in transgenic mice, a constitutive activation of PPARα in mammary gland alters its development and the lactation leading to mortality of neonates [7]. Furthermore, mice with a deletion of PPARγ in mammary gland produce a "toxic milk" containing elevated levels of inflammatory lipids. Despite these strong phenotypes, the mechanisms of action of these receptors remain unclear in the control of fertility and further investigations are needed to better use them in medical treatments.

In the future, these drugs might also be used in a large spectrum of treatments targeting reproduction such as improvement of follicular development, in vitro fertilization, certain complications of pregnancy, and sex hormone-sensitive cancers affecting the reproductive tissues, including breast, prostate, ovary, or cancers affecting pituitary cells (pituitary adenomas). For example, new generation of pharmacological drugs targeting these receptors are already in clinical use or are undergoing testing for use as therapeutic agents. Synthetic molecules (glitazone molecules, which bind to PPARα, or glitazar molecules, which bind to PPARα/PPARγ), currently being tested in clinical studies, may prove particularly useful for the treatment of certain types of infertility associated with metabolism disorders such as insulinoresistance in polycystic ovary syndrome (PCOS) [8]. The therapeutical treatment in women with pregnancy-specific diseases could be also changed. Women with severe preeclampsia have a reduction in serum levels of PPAR activating lipids several weeks before the onset of symptoms. The use of PPAR ligands might be proposed to ameliorate the disorders associated with preeclampsia such as hypertension and inflammation. Moreover, the rate of success of in vitro fertilization (IVF) could be improved in the next decade

by addition in the culture media of PPARβ/δ ligands. Recent studies have shown that the development and implantation of IVF embryos may be augmented by supplementing culture media with PGI2 analogs, a synthetic PPARβ/δ ligand, or retinoic acid [9]. Of note, in all these examples, potential long-term adverse effects are unknown, and more data need to be acquired to consider these drugs "safe" during pregnancy.

Finally, in this issue, we report the putative functions of PPARs and RXRs in gonads, placenta, and embryo and we will discuss the possible role of PPARs as mediators of environmental toxicity for reproductive function.

P. Froment

REFERENCES

[1] C. M. Komar, "Peroxisome proliferator-activated receptors (PPARs) and ovarian function—implications for regulating steroidogenesis, differentiation, and tissue remodeling," *Reproductive Biology and Endocrinology*, pp. 3–41, 2005.

[2] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[3] Y. Cui, K. Miyoshi, E. Claudio, et al., "Loss of the peroxisome proliferation-activated receptor gamma (PPARγ) does not affect mammary development and propensity for tumor formation but leads to reduced fertility," *The Journal of Biological Chemistry*, vol. 277, no. 20, pp. 17830–17835, 2002.

[4] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2001.

[5] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPAR γ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[6] O. Wendling, P. Chambon, and M. Mark, "Retinoid X receptors are essential for early mouse development and placentogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 2, pp. 547–551, 1999.

[7] Q. Yang, R. Kurotani, A. Yamada, S. Kimura, and F. J. Gonzalez, "Peroxisome proliferator-activated receptor alpha activation during pregnancy severely impairs mammary lobuloalveolar development in mice," *Endocrinology*, vol. 147, no. 10, pp. 4772–4780, 2006.

[8] P. Froment and P. Touraine, "Thiazolidinediones and fertility in polycystic ovary syndrome (PCOS)," *PPAR Research*, vol. 2006, Article ID 73986, 8 pages, 2006.

[9] J. C. Huang, W. S. Wun, J. S. Goldsby, I. C. Wun, D. Noorhasan, and K. K. Wu, "Stimulation of embryo hatching and implantation by prostacyclin and peroxisome proliferator-activated receptor δ activation: implication in IVF," *Human Reproduction*, vol. 22, no. 3, pp. 807–814, 2007.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 723243, 8 pages
doi:10.1155/2008/723243

Review Article

PPARs and Female Reproduction: Evidence from Genetically Manipulated Mice

Jichun Yang, Lihong Chen, Xiaoyan Zhang, Yunfeng Zhou, Dongjuan Zhang, Ming Huo, and Youfei Guan

Department of Physiology and Pathophysiology, Peking (Beijing) University Diabetes Center, Peking (Beijing) University Health Science Center, Beijing 100083, China

Correspondence should be addressed to Youfei Guan, youfeiguan@bjmu.edu.cn

Received 1 July 2007; Accepted 6 December 2007

Recommended by Pascal Froment

Peroxisome proliferator-activated receptors (PPARs) are ligand-activated nuclear receptors controlling many important physiological processes, including lipid and glucose metabolism, energy homeostasis, inflammation, as well as cell proliferation and differentiation. In the past decade, intensive study of PPARs has shed novel insight into prevention and treatment of dyslipidemia, insulin resistance, and type 2 diabetes. Recently, a large body of research revealed that PPARs are also functionally expressed in reproductive organs and various parts of placenta during pregnancy, which strongly suggests that PPARs might play a critical role in reproduction and development, in addition to their central actions in energy homeostasis. In this review, we summarize recent findings elucidating the role of PPARs in female reproduction, with particular focus on evidence from gene knockout and transgenic animal model study.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are members of the ligand-activated nuclear hormone receptor superfamily of 49 members that participate in many physiological functions [1]. To date, three isotypes, designated as PPARα, PPARβ/δ, and PPARγ, have been identified in many species, including frogs, rodents, and humans [2, 3]. PPARα is highly expressed in liver, kidney, heart, skeletal muscle, and other tissues involving fatty acid oxidation and it had been demonstrated to be the central regulator of fatty acid β-oxidation, fatty acid (FA) transport, and lipoprotein synthesis in these tissues. Activation of PPARα by its natural or synthetic ligands enhances FA uptake and oxidation in liver, which is beneficial for ameliorating dyslipidemia [4, 5]. PPARγ is predominantly expressed in adipose tissue and is a key regulator of adipocyte differentiation and triglyceride storage, whereas PPARβ/δ is ubiquitously expressed in almost all tissues and believed to be involved in lipid metabolism [4, 6]. In contrast to intensive research into PPARγ and PPARα, little exists for PPARβ/δ. After binding by their endogenous ligands, such as 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (15dPGJ2) and long-chain FAs, or exogenous synthetic agonists, such as thiazolidinediones (TZDs) and fibrates, PPARs will heterodimerize with another nuclear receptor called retinoid X receptor alpha (RXRα). The PPARs/RXRα heterodimer binds to a specific DNA sequence called PPAR-responsive element (PPRE) located in promoter regions of the target genes to initiate or silence gene transcription. A typical PPRE consists of a repeat AGGTCA separated by one nucleotide. However, activation of PPARs is far more complex than this, with complicated cross-talk among PPARs, RXRs, ligands, corepressors, coactivators, and many other factors [7, 8].

Because PPARs play key roles in regulating energy homeostasis, particularly FA oxidation and carbohydrate metabolism, numerous studies have been conducted in the past decade to develop synthetic PPAR agonists for therapeutic treatment of metabolic diseases, including dyslipidemia, insulin resistance, and type 2 diabetes. Long before being identified as PPARα agonists, fibrates were clinically prescribed for treatment of dyslipidemia. Subsequently, TZDs, structural analogues of fibrates, were shown to selectively activate PPARγ [7, 9–11]. To date, several TZDs, including pioglitazone and rosiglitazone, improve glycemic control in patients with type 2 diabetes or glucose intolerance via their insulin-sensitizing activity, mainly achieved by preventing FA uptake and adipose deposition in insulin-sensitive tissues

such as liver, muscle, and pancreas [7, 9–11]. In addition, potent agonists for activation of multiple PPAR isotypes now in development, such as dual PPARα/γ agonists, have considerable promise for improving glycemic control with fewer side effects. As well, PPARβ/δ agonists are currently under development.

The nutrients glucose and FA and fuel sensors insulin and leptin have long been known to be critical in regulating female reproduction [12–14]. During the onset of puberty, molecules such as leptin and neuropeptide Y might function as energy sensors and initiate reproduction processes under conditions of sufficient body energy storage [13, 15, 16]. Given the well-documented central roles of PPARs in energy homeostasis and because energy status is directly linked to reproduction [13, 14], it is reasonable to speculate that PPARs may play important roles in female reproduction. In fact, many recent studies have examined the potential role of PPARs in reproduction. In rodents, PPAR knockout mouse models have provided direct evidence of a critical role of PPARs in reproduction and placenta development (Table 1). PPARγ-null mouse fetuses were shown to die by embryonic day 10 because of failed formation of the vascular labyrinth [17, 18], and PPARβ/δ-null mice also exhibited abnormal placenta during development [19]. In contrast to PPARγ- and PPARβ/δ-null mice, PPARα-null mice displayed no placental abnormality but, rather, increased risk of maternal abortion and offspring neonatal mortality [20]. Subsequent studies involving RT-PCR, in situ hybridization, immunohistochemistry, and Northern and Western blot analysis further revealed all three PPAR isotypes are expressed in reproductive tissues such as testis (sperm), ovary (oocyte), as well as various parts of the placenta of rat, mouse, and human [12, 21, 22]. Importantly, pregnant rats given oral troglitazone showed significantly increased placental PPARγ expression as well as reduced mortality of fetuses by about 50% [23]. Loss-of-function mutations of PPARs have provided excellent models for studying the roles of PPARs in human reproduction and placenta development. To date, three groups of loss-of-function mutations of PPARγ have been described [6, 24–26]. In one study, about 40% of female subjects with loss-of-function mutations of PPARγ had polycystic ovary syndrome (PCOS) [6], which has been believed to be associated with infertility in women. Consistent with these observations, administration of insulin-sensitizers TZDs and metformin improved ovulation function and fertility and enhanced growth hormone (GH) secretion in women with PCOS [27, 28]. Collectively, these findings imply an important role for PPARs in mammalian reproduction.

In this review, we discuss PPARs expression in female reproductive tissues and their roles in female reproduction, with a focus on genetically manipulated mice.

2. PPARs: TISSUE DISTRIBUTION IN FEMALE REPRODUCTIVE SYSTEM

2.1. Hypothalamic-pituitary axis

All three PPAR isotypes have been detected in the mouse pituitary gland [29]. PPARγ is highly expressed in normal human pituitary gland and in all normal pituitary secreting cell lines [30]. Because of its antiproliferative effects in pituitary cells, activation of PPARγ by TZDs inhibited the development of pituitary adenomas in mice and humans [31]. Despite its presence in the hypothalamic-pituitary axis, the precise roles of PPARγ in reproductive cells remain poorly understood. Although PPARγ expression is evident in pituitary tissue, TZD treatment failed to affect the in vitro secretion of ovine pituitary hormones, including prolactin (PRL), growth hormone (GH), follicle-stimulating hormone (FSH), and luteinizing hormone (LH), and also no alteration of the LH secretion was observed in LbetaT2 cells, a murine gonadotropic pituitary tumor cell line [12].

2.2. Ovary

All three isotypes of PPAR are expressed in ovarian tissue. PPARα and PPARβ/δ are expressed primarily in the theca and stroma tissues [32], whereas PPARγ, more extensively studied, was detected in ovaries of mouse, rat, pig, sheep, cow, and human. In the ovaries of rodents and ruminants, PPARγ is highly expressed in granulosa cells, with lower expression in theca cells and the corpus luteum [12]. In humans, PPARγ was present in granulosa cells collected during oocyte aspiration from women undergoing treatment for in vitro fertilization [33]. Unlike the constant expression of PPARα and PPARβ/δ throughout follicular development and the ovarian cycle, the expression of PPARγ is downregulated in response to LH surge. PPARγ expression seems to be tightly regulated in the ovary, and its regulatory expression is the primary mechanism by which LH influences the activity of PPARγ [34].

2.3. Uterus and placenta

Although all three PPAR isotypes are functionally expressed in uterus, they display different expression profiles with the development of placenta in pregnancy [35–37]. In endometria of ewe, PPARα expression declines between day 7 and day 17 of pregnancy, whereas PPARβ/δ is constantly expressed at all developmental stages and PPARγ expression is erratically regulated. In addition, RXRs differ from that of PPARs, which suggests that different PPAR/RXR heterodimers might form and function distinctly as development proceeds [35]. All three PPAR isotypes have been reported in placenta in rodents and humans. PPARγ was the first to be detected in a human choriocarcinoma-derived JEG cell line by Northern blot analysis [34]. In human placenta, PPARγ is expressed in early and term villous trophoblasts and in extravillous trophoblasts in first-trimester placentas [21]. PPARγ was also detected in mouse placenta as early as embryonic day 8.5 [38] and in rat placenta by day 11 [23]. In mice, PPARγ is expressed in spongiotrophoblasts and in the vascular labyrinth that forms the interface between maternal and fetal circulation to control nutrient exchange [23]. In rodent placenta, PPARα and PPARβ/δ are present in the junctional zone, which has invasive and endocrine functions, and in the labyrinth, whereas in human placenta, they are in villous trophoblasts, particularly syncytiotrophoblasts [39]. However,

in cultured villous trophoblasts of human term placenta, PPARα and PPARβ/δ transcript levels were higher in cytotrophoblasts than in syncytiotrophoblasts [40].

2.4. Mammary gland

All three isotypes of PPAR are detected in rodent mammary gland and human breast cell lines [41–44]. During pregnancy and lactation, the PPARα and γ mRNAs decreased while the PPARβ/δ mRNA remained relatively unchanged in mouse mammary gland [41].

3. PPARα AND FEMALE REPRODUCTION

During pregnancy, placental transfer of FA and other nutrients from the mother to the fetus is crucial for adequate fetal growth and development, and PPARα might play a crucial role in this process because of its central role in FA transport and oxidation [4, 10, 39]. Recently, gemfibrozil and clofibrate, two PPARα agonists, were shown to downregulate human chorionic gonadotrophin and upregulate progesterone secretion in human trophoblasts, which suggests that activation of PPARα might be beneficial for the secretion of these hormones, so essential for maintaining pregnancy [45]. More recently, evidence for a key role of PPARα in placenta development was demonstrated by increased abortion rate (by 20%) in PPARα-null mice without diabetes [4, 20]. In PPARα-null mice with diabetes, the mean abortion rate was approximately 50%, as compared with 8.3% for wild-type mice. Moreover, PPARα-null mice showed higher neonatal mortality than wild-type mice: for mice without diabetes, the rate was 13.3% versus 5.1%, respectively, and for mice with diabetes, 78.9% versus 27.7% [20]. Thus, PPARα might have an important role in maternal-fetal nutrient exchange, and its deficiency could be deleterious to fetal development. This study further supported that tight control of blood glucose is beneficial for improving the fertility of diabetic women and, as clearly indicated in this study, abortion rate and neonatal mortality were increased in both wild-type and PPARα-null mice with diabetes.

Controversially, some other reports indicated that activation of PPARα might be deleterious to development of female reproductive cells. PPARα can bind to estrogen response elements and act as a competitive inhibitor of estrogen receptor [46, 47]. Activation of PPARα decreased the expression and activity of aromatase in granulosa cells [48], thus resulting in decreased estradio synthesis. More recently, treatment with the PPARα agonist fenofibrate decreased the level of aromatase in wild-type mice but enhanced it in PPARα-null mice [49]. A critical role for PPARα in mammary gland function was supported by a recent study in which transgenic mice expressing a constitutively activated PPARα form (VP16PPARα) in the stratified epithelia had a severe defect in mammary gland development and lactation during pregnancy, resulting in 100% neonate mortality [50]. Taken together, these observations reveal that PPARα plays an important role in mammalian female reproduction, but further research work is required to clarify its definite role and underlying molecular mechanism(s).

4. PPARβ/δ AND FEMALE REPRODUCTION

PPARβ/δ is ubiquitously expressed in the ovary at a constant level during the estrous cycle and pseudopregnancy [51], which suggests that PPARβ/δ may be involved in normal ovarian function in theca, stroma, and luteal cells. One study showed that PPARβ/δ mRNA was almost absent on mouse embryo days 1–4 but was significantly expressed in the subluminal stroma surrounding blastocysts on day 5, just after embryo implantation. Subsequently, PPARβ/δ expression was increased in the decidua on days 6–8 [36, 52]. A similar process was observed in rat as well, intense PPARβ/δ immunostaining was observed in rat decidua under artificial decidualization but not in uninjected control horns [53]. These data suggest that PPARβ/δ expression at implantation sites requires an active blastocyst or analog and may play an essential role in blastocyst implantation.

A large body of research has indicated that PPARβ/δ mediates the important role of COX-2-derived prostaglandin I2 (prostacyclin, PGI2) in pregnancy. COX-2 knockout female mice displayed decreased fertility, in part due to deficiency of blastocyte implantation and decidualization [52, 54]. Treatment of these mice with a PGI2 analogue, carboprostacyclin, or the PPARβ/δ-selective agonist L-165041 restored implantation [52]. PGI2 is the most abundant prostaglandin at implantation sites where PPARβ/δ and COX-2 were colocalized and strongly upregulated during pregnancy in a similar manner [52]. As a potent endogenous PPARβ/δ ligand, PGI2 can act as a vasoactive agent to increase vascular permeability [55, 56] and blastocyst hatching [57], so the high expression of PPARβ/δ in the subluminal stroma at implantation sites might mediate this process, facilitating the implantation of the embryo [58]. This suggestion was further confirmed by placentas of PPARβ/δ-null mice displaying abnormal vascular development [19] and that giant-cell differentiation of placentas requires an intact PPARβ/δ signaling pathway [57].

In addition to the important roles of PPARβ/δ at implantation sites of the maternal body, the expression and function of PPARβ/δ in the embryo are of interest. Compared to the development of in vivo embryos, cultured embryos, such as in vitro fertilization (IVF) embryos, are retarded because they lack the protective environment of the maternal body [59]. Supplementing culture media with milepost, a stable analog of PGI2, enhanced mouse blastocyst hatching [60]. Recent work showed that preimplantation embryos express PPARβ/δ, which is essential for the enhancing effect of PGI2 and the spontaneous progression of the embryos. PGI2 promoted the development of wild-type embryos in vitro and enhanced their implantation potential but had no effect on PPARβ/δ-null embryos [61].

PPARβ/δ is expressed ubiquitously at higher levels during embryogenesis than in adulthood [62, 63]. In addition, homozygous loss of PPARβ/δ caused frequent embryonic lethality, but surviving PPARβ/δ-deficient offspring did not die postnatally, which suggests that the essential function of the receptor is restricted to the gestational period [19].

Given the roles of PPARβ/δ in embryo development and implantation, the activity of PPARβ/δ agonists under

Table 1: Studies of reproductive phenotypes of female PPARα, PPARβ/δ, and PPARγ-null or transgenic mice.

PPAR isotype		Reproductive phenotypes	References
PPARα	KO	Maternal abortion and neonatal death; altered ovarian estradiol production	Yessoufou et al. [20], Lefebvre et al. [4]
	TG	Defect in mammary gland development; defect in lactation during pregnancy	Yang et al. [50]
PPARβ/δ	KO	Placental defects; frequent (>90%) midgestation lethality; placenta lipid accumulation defects	Barak et al. [19], Nadra et al. [57]
PPARγ	KO	Embryonic death at embryo day 10; embryonic lipid droplets lacking; placental malformed labyrinth zone; toxic milk	Barak et al. [17], Kubota et al. [18], Wan et al. [71]
	TG	Exacerbates mammary gland tumor development	Saez et al. [73]

KO: global or tissue-specific knockout; TG: tissue-specific transgenic.

development should be carefully evaluated to avoid possible complications in pregnancy with their use.

5. PPARγ AND FEMALE REPRODUCTION

After ovulation, the expression of PPARγ in the corpus luteum increases, otherwise the corpus luteum regresses and PPARγ expression decreases if no fertilization or embryo implantation occurs [64, 65]. Thus, PPARγ might play a role in fertility control. Indeed, mice with specific deletion of PPARγ in granulosa cells exhibited reduced fertility [66]. Luteal expression of PPARγ might be important for the pregnancy, possibly via maintaining production of progesterone to support implantation and gestation [67].

PPARγ-null embryos were shown to die by embryonic day 10 [17], as a result of placenta alteration and malformed vascular labyrinth due to PPARγ deficiency, which disrupts the interface between trophoblasts and the fetal endothelium and leads to embryonic myocardial thinning. A tetraploid-rescued mutant overcame the placenta defect for survival to term. Consistent with this observation, an RXRα-(PPARγ hetero-partner) or RXRα/RXRβ-null mutant exhibited a similar phenotype to that of PPARγ-null mice [17, 68]. The expression of Mucin 1 (MUC1), a PPARγ target gene, is lost in PPARγ-null mice, whereas its expression in wild-type mice can be upregulated by PPARγ agonist treatment. MUC1 expressed in the apical surface of the labyrinth helps in differentiation of trophoblast stem cells and invokes developmental and functional analogies between the placental blood sinuses and luminal epithelia [69].

During early term pregnancy, placental trophoblasts invade the uterine wall and establish the maternal-fetal exchange. PPARγ plays a dominant role in this process. The differentiation of the placenta is characterized by fusion of cytotrophoblasts into syncytiotrophoblasts, which are more resistant than cytotrophoblasts to hypoxic injury. Activation of PPARγ stimulates this differentiation process [21]. PPARγ agonists increase FA uptake and adipose accumulation in trophoblasts [70], and PPARγ-null or RXRα-null murine embryos show fewer lipid droplets than wild-type embryos [17, 68], which suggests an important role of PPARγ in providing sufficient nutrients for embryo development. Moreover, it is indicated in one latest study that PPARγ deletion in mammary gland resulted in the production of "toxic milk" containing elevated levels of inflammatory lipids, which results in inflammation, alopecia, and growth retardation in the nursing neonates [71]. Peroxisome proliferator-activated receptor-binding protein (PBP) serves as an anchor for recruiting PPAR mediator complexes, and is necessary for activation of PPARs. Moreover, specific knockout of PBP in mouse mammary gland resulted in a severe defect in mammary gland development, indeed the PBP-null mammary gland failed to produce milk for nursing neonates during lactation [72]. These studies clearly indicated that PPARγ/PPAR-binding protein expression are also vital for providing high-quality milk for nursing the neonates and protecting them from inflammatory lipids [71]. Interestingly and unexpectedly, constitutive expression of an active form of PPARγ (Vp16PPARγ) in mammary gland exacerbated mammary gland tumor development via enhanced Wnt signaling [73].

Proinflammatory proteins and cytokines are associated with term and preterm labor and stimulate uterine contraction [74]; PPARγ might be implicated in this process because of its ability to suppress inflammatory cytokine secretion [75]. The natural ligands of placental PPARγ may be present in maternal circulation, which could be naturally occurring prostanoids or FAs and some reproductive hormones. This hypothesis is supported by the observation that serum from pregnant women activated PPARγ expression in JEG-3 cells, while serum from nonpregnant women having no such effect [76].

In addition, as a target gene of PPARγ, another nuclear receptor, liver X receptor (LXR), participates in regulation of female reproduction. The two isforms, α and β, both act as transcription factors activated by binding of specific cholesterol metabolites [77]. LXRs play important roles in many metabolic pathways, such as cholesterol, lipid, and carbohydrate metabolism. In addition to these regulatory actions, LXRs affect reproductive function. Mice deficient in LXRα, LXRβ, or both showed decreased ability to conceive and fewer pups per litter as compared with wild-type mice [78]. As well, both LXRα and β are expressed in mouse oocytes and seem to affect ovarian function [78]. Lipid distribution in the uterus plays a critical role for its function. LXR prevents accumulation of cholesteryl esters in the mouse myometrium

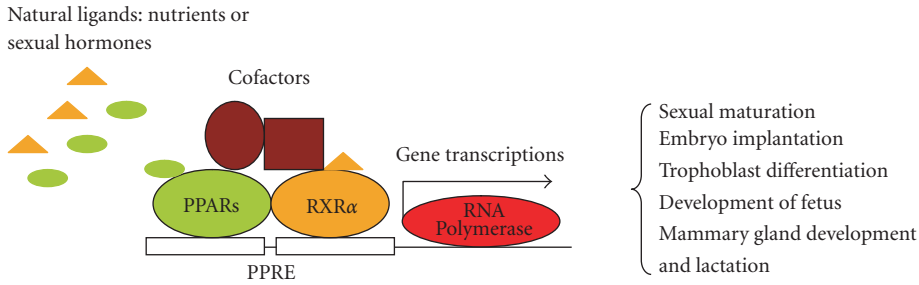


Figure 1: Schematic presentation of regulatory roles of PPARs in reproduction and development.

by controlling the expression of genes (ABCA1 and ABCG1) involved in cholesterol efflux and storage. As well, mice lacking LXRβ showed a contractile activity defect induced by oxytocin or PGF2α [79]. Taken together, gene knockout results suggest that PPARγ/LXR might participate in embryonic development by sensing changes in levels of nutrients, hormones, and/or other signals.

6. CONCLUSION

A large body of research has revealed that in addition to their central roles in regulating FA oxidation and glucose homeostasis, PPARs are highly expressed in reproductive tissues and placenta, so PPARs might also be key regulators of reproduction and development (Table 1). At the early stage of sexual maturation, PPARs might be activated in response to energy status and/or circulating hormones for involvement in maturation of reproductive cells. During gestation, PPARs are highly expressed in trophoblasts and directly involved in cytotrophoblast differentiation and function, possibly functioning as energy-signal sensors and transporters for nutrients and gases between maternal and fetus circulation to provide sufficient nutrients for development of the fetus (see Figure 1). Moreover, PPARs also play important roles in mammary gland development and maternal PPARs are vital for producing high-quality milk for nursing neonates. However, further research is required to address the following questions. (1) What are the natural ligands for activation of PPARs in reproduction and development, nutrients, sexual hormones, or other factors? (2) What are the underlying molecular mechanisms of PPAR activation in response to their natural ligands? Given the critical roles of all three PPAR isotypes in female reproduction, caution should be taken in the clinical use of PPARα and PPARγ agonists in young women.

ACKNOWLEDGMENTS

The authors thank Dr. Jing Li and Dr. Dan Pu for their assistance in manuscript preparation. This work was supported by grants from the Natural Science Foundation of China (Grant no. NSFC 30670766, 30530340, 30771030) and the Ministry of Science and Technology of China (Grant no. 2006CB503907 to Y. Guan).

REFERENCES

[1] X. Yang, M. Downes, R. Yu, et al., "Nuclear receptor expression links the circadian clock to metabolism," *Cell*, vol. 126, no. 4, pp. 801–810, 2006.

[2] M. Lehrke and M. A. Lazar, "The many faces of PPARγ," *Cell*, vol. 123, no. 6, pp. 993–999, 2005.

[3] P. Balakumar, M. Rose, and M. Singh, "PPAR ligands: are they potential agents for cardiovascular disorders?" *Pharmacology*, vol. 80, no. 1, pp. 1–10, 2007.

[4] P. Lefebvre, G. Chinetti, J.-C. Fruchart, and B. Staels, "Sorting out the roles of PPARα in energy metabolism and vascular homeostasis," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 571–580, 2006.

[5] S. W. Beaven and P. Tontonoz, "Nuclear receptors in lipid metabolism: targeting the heart of dyslipidemia," *Annual Review of Medicine*, vol. 57, pp. 313–329, 2006.

[6] R. K. Semple, V. K. Chatterjee, and S. O'Rahilly, "PPARγ and human metabolic disease," *Journal of Clinical Investigation*, vol. 116, no. 3, pp. 581–589, 2006.

[7] Y. Zhang and Y. Guan, "PPAR-γ agonists and diabetic nephropathy," *Current Diabetes Reports*, vol. 5, no. 6, pp. 470–475, 2005.

[8] V. Zoete, A. Grosdidier, and O. Michielin, "Peroxisome proliferator-activated receptor structures: ligand specificity, molecular switch and interactions with regulators," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 915–925, 2007.

[9] B. Staels and J.-C. Fruchart, "Therapeutic roles of peroxisome proliferator-activated receptor agonists," *Diabetes*, vol. 54, no. 8, pp. 2460–2470, 2005.

[10] Y. Guan, "Peroxisome proliferator-activated receptor family and its relationship to renal complications of the metabolic syndrome," *Journal of the American Society of Nephrology*, vol. 15, no. 11, pp. 2801–2815, 2004.

[11] G. Wang, J. Wei, Y. Guan, N. Jin, J. Mao, and X. Wang, "Peroxisome proliferator-activated receptor-γ agonist rosiglitazone reduces clinical inflammatory responses in type 2 diabetes with coronary artery disease after coronary angioplasty," *Metabolism*, vol. 54, no. 5, pp. 590–597, 2005.

[12] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[13] A. Cervero, F. Domínguez, J. A. Horcajadas, A. Quiñonero, A. Pellicer, and C. Simón, "The role of the leptin in reproduction," *Current Opinion in Obstetrics & Gynecology*, vol. 18, no. 3, pp. 297–303, 2006.

[14] The ESHRE Capri Workshop Group, "Nutrition and reproduction in women," *Human Reproduction Update*, vol. 12, no. 3, pp. 193–207, 2006.

[15] W. Kiess, W. F. Blum, and M. L. Aubert, "Leptin, puberty and reproductive function: lessons from animal studies and observations in humans," *European Journal of Endocrinology*, vol. 138, no. 1, pp. 26–29, 1998.

[16] W. Kiess, A. Reich, K. Meyer, et al., "A role for leptin in sexual maturation and puberty?" *Hormone Research*, vol. 51, supplement 3, pp. 55–63, 1999.

[17] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[18] N. Kubota, Y. Terauchi, H. Miki, et al., "PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance," *Molecular Cell*, vol. 4, no. 4, pp. 597–609, 1999.

[19] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[20] A. Yessoufou, A. Hichami, P. Besnard, K. Moutairou, and N. A. Khan, "Peroxisome proliferator-activated receptor α deficiency increases the risk of maternal abortion and neonatal mortality in murine pregnancy with or without diabetes mellitus: modulation of T cell differentiation," *Endocrinology*, vol. 147, no. 9, pp. 4410–4418, 2006.

[21] T. Fournier, V. Tsatsaris, K. Handschuh, and D. Evain-Brion, "PPARs and the placenta," *Placenta*, vol. 28, no. 2-3, pp. 65–76, 2007.

[22] W. T. Schaiff, Y. Barak, and Y. Sadovsky, "The pleiotropic function of PPARγ in the placenta," *Molecular and Cellular Endocrinology*, vol. 249, no. 1-2, pp. 10–15, 2006.

[23] R. Asami-Miyagishi, S. Iseki, M. Usui, K. Uchida, H. Kubo, and I. Morita, "Expression and function of PPARγ in rat placental development," *Biochemical and Biophysical Research Communications*, vol. 315, no. 2, pp. 497–501, 2004.

[24] I. Barroso, M. Gurnell, V. E. F. Crowley, et al., "Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension," *Nature*, vol. 402, no. 6764, pp. 880–883, 1999.

[25] A. K. Agarwal and A. Garg, "A novel heterozygous mutation in peroxisome proliferator-activated receptor-γ gene in a patient with familial partial lipodystrophy," *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 1, pp. 408–411, 2002.

[26] R. A. Hegele, H. Cao, C. Frankowski, S. T. Mathews, and T. Leff, "PPARG F388L, a transactivation-deficient mutant, in familial partial lipodystrophy," *Diabetes*, vol. 51, no. 12, pp. 3586–3590, 2002.

[27] D. Glintborg, R. K. Støving, C. Hagen, et al., "Pioglitazone treatment increases spontaneous growth hormone (GH) secretion and stimulated GH levels in polycystic ovary syndrome," *Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 10, pp. 5605–5612, 2005.

[28] E. Seli and A. J. Duleba, "Optimizing ovulation induction in women with polycystic ovary syndrome," *Current Opinion in Obstetrics & Gynecology*, vol. 14, no. 3, pp. 245–254, 2002.

[29] A. L. Bookout, Y. Jeong, M. Downes, R. T. Yu, R. M. Evans, and D. J. Mangelsdorf, "Anatomical profiling of nuclear receptor expression reveals a hierarchical transcriptional network," *Cell*, vol. 126, no. 4, pp. 789–799, 2006.

[30] F. Bogazzi, D. Russo, M. T. Locci, et al., "Peroxisome proliferator-activated receptor (PPAR)γ is highly expressed in normal human pituitary gland," *Journal of Endocrinological Investigation*, vol. 28, no. 10, pp. 899–904, 2005.

[31] A. P. Heaney, M. Fernando, and S. Melmed, "PPAR-γ receptor ligands: novel therapy for pituitary adenomas," *Journal of Clinical Investigation*, vol. 111, no. 9, pp. 1381–1388, 2003.

[32] S. S. Lee, T. Pineau, J. Drago, et al., "Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators," *Molecular and Cellular Biology*, vol. 15, no. 6, pp. 3012–3022, 1995.

[33] C. M. Komar, "Peroxisome proliferator-activated receptors (PPARs) and ovarian function—implications for regulating steroidogenesis, differentiation, and tissue remodeling," *Reproductive Biology and Endocrinology*, vol. 3, p. 41, 2005.

[34] J. Banerjee and C. M. Komar, "Effects of luteinizing hormone on peroxisome proliferator-activated receptor γ in the rat ovary before and after the gonadotropin surge," *Reproduction*, vol. 131, no. 1, pp. 93–101, 2006.

[35] L. Cammas, P. Reinaud, N. Bordas, O. Dubois, G. Germain, and G. Charpigny, "Developmental regulation of prostacyclin synthase and prostacyclin receptors in the ovine uterus and conceptus during the peri-implantation period," *Reproduction*, vol. 131, no. 5, pp. 917–927, 2006.

[36] N.-Z. Ding, C.-B. Teng, H. Ma, et al., "Peroxisome proliferator-activated receptor δ expression and regulation in mouse uterus during embryo implantation and decidualization," *Molecular Reproduction and Development*, vol. 66, no. 3, pp. 218–224, 2003.

[37] E. Lord, B. D. Murphy, J. A. Desmarais, S. Ledoux, D. Beaudry, and M.-F. Palin, "Modulation of peroxisome proliferator-activated receptor δ and γ transcripts in swine endometrial tissue during early gestation," *Reproduction*, vol. 131, no. 5, pp. 929–942, 2006.

[38] A. Cutando and J. A. Gil-Montoya, "The dental patient with hemostatic disorders. A review of hemostasia physiopathology for dental professionals," *Medicina Oral*, vol. 4, no. 3, pp. 485–493, 1999.

[39] Q. Wang, H. Fujii, and G. T. Knipp, "Expression of PPAR and RXR isoforms in the developing rat and human term placentas," *Placenta*, vol. 23, no. 8-9, pp. 661–671, 2002.

[40] G. Daoud, L. Simoneau, A. Masse, E. Rassart, and J. Lafond, "Expression of cFABP and PPAR in trophoblast cells: effect of PPAR ligands on linoleic acid uptake and differentiation," *Biochimica et Biophysica Acta*, vol. 1687, no. 1–3, pp. 181–194, 2005.

[41] J. M. Gimble, G. M. Pighetti, M. R. Lerner, et al., "Expression of peroxisome proliferator activated receptor mRNA in normal and tumorigenic rodent mammary glands," *Biochemical and Biophysical Research Communications*, vol. 253, no. 3, pp. 813–817, 1998.

[42] K. M. Suchanek, F. J. May, W. Jae Lee, N. A. Holman, and S. J. Roberts-Thomson, "Peroxisome proliferator-activated receptor β expression in human breast epithelial cell lines of tumorigenic and non-tumorigenic origin," *The International Journal of Biochemistry & Cell Biology*, vol. 34, no. 9, pp. 1051–1058, 2002.

[43] K. M. Suchanek, F. J. May, J. A. Robinson, et al., "Peroxisome proliferator-activated receptor α in the human breast cancer cell lines MCF-7 and MDA-MB-231," *Molecular Carcinogenesis*, vol. 34, no. 4, pp. 165–171, 2002.

[44] D. Bonofiglio, S. Aquila, S. Catalano, et al., "Peroxisome proliferator-activated receptor-γ activates p53 gene promoter binding to the nuclear factor-κB sequence in human MCF7 breast cancer cells," *Molecular Endocrinology*, vol. 20, no. 12, pp. 3083–3092, 2006.

[45] F. Hashimoto, Y. Oguchi, M. Morita, et al., "PPARα agonists clofibrate and gemfibrozil inhibit cell growth, down-regulate hCG and up-regulate progesterone secretions in immortalized human trophoblast cells," *Biochemical Pharmacology*, vol. 68, no. 2, pp. 313–321, 2004.

[46] H. Keller, F. Givel, M. Perroud, and W. Wahli, "Signaling cross-talk between peroxisome proliferator-activated receptor/retinoid X receptor and estrogen receptor through estrogen response elements," *Molecular Endocrinology*, vol. 9, no. 7, pp. 794–804, 1995.

[47] S. B. Nuñez, J. A. Medin, O. Braissant, et al., "Retinoid X receptor and peroxisome proliferator-activated receptor activate an estrogen responsive gene independent of the estrogen receptor," *Molecular and Cellular Endocrinology*, vol. 127, no. 1, pp. 27–40, 1997.

[48] T. Lovekamp-Swan, A. M. Jetten, and B. J. Davis, "Dual activation of PPARα and PPARγ by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells," *Molecular and Cellular Endocrinology*, vol. 201, no. 1-2, pp. 133–141, 2003.

[49] K. Toda, T. Okada, C. Miyaura, and T. Saibara, "Fenofibrate, a ligand for PPARα, inhibits aromatase cytochrome P450 expression in the ovary of mouse," *Journal of Lipid Research*, vol. 44, no. 2, pp. 265–270, 2003.

[50] Q. Yang, R. Kurotani, A. Yamada, S. Kimura, and F. J. Gonzalez, "Peroxisome proliferator-activated receptor α activation during pregnancy severely impairs mammary lobuloalveolar development in mice," *Endocrinology*, vol. 147, no. 10, pp. 4772–4780, 2006.

[51] C. M. Komar and T. E. Curry Jr., "Localization and expression of messenger RNAs for the peroxisome proliferator-activated receptors in ovarian tissue from naturally cycling and pseudopregnant rats," *Biology of Reproduction*, vol. 66, no. 5, pp. 1531–1539, 2002.

[52] H. Lim, R. A. Gupta, W.-G. Ma, et al., "Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ," *Genes & development*, vol. 13, no. 12, pp. 1561–1574, 1999.

[53] N.-Z. Ding, X-H. Ma, H-L. Diao, L-B. Xu, and Z-M. Yang, "Differential expression of peroxisome proliferator-activated receptor δ at implantation sites and in decidual cells of rat uterus," *Reproduction*, vol. 125, no. 6, pp. 817–825, 2003.

[54] H. Lim, B. C. Paria, S. K. Das, et al., "Multiple female reproductive failures in cyclooxygenase 2-deficient mice," *Cell*, vol. 91, no. 2, pp. 197–208, 1997.

[55] C. Wheeler-Jones, R. Abu-Ghazaleh, R. Cospedal, R. A. Houliston, J. Martin, and I. Zachary, "Vascular endothelial growth factor stimulates prostacyclin production and activation of cytosolic phospholipase A_2 in endothelial cells via p42/p44 mitogen-activated protein kinase," *FEBS Letters*, vol. 420, no. 1, pp. 28–32, 1997.

[56] T. Murohara, J. R. Horowitz, M. Silver, et al., "Vascular endothelial growth factor/vascular permeability factor enhances vascular permeability via nitric oxide and prostacyclin," *Circulation*, vol. 97, no. 1, pp. 99–107, 1998.

[57] K. Nadra, S. I. Anghel, E. Joye, et al., "Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor β/δ," *Molecular and Cellular Biology*, vol. 26, no. 8, pp. 3266–3281, 2006.

[58] A. Psychoyos, "Uterine receptivity for nidation," *Annals of the New York Academy of Sciences*, vol. 476, no. 1, pp. 36–42, 1986.

[59] K. Hardy, "Cell death in the mammalian blastocyst," *Molecular Human Reproduction*, vol. 3, no. 10, pp. 919–925, 1997.

[60] J.-C. Huang, W.-S. A. Wun, J. S. Goldsby, I. C. Wun, S. M. Falconi, and K. K. Wu, "Prostacyclin enhances embryo hatching but not sperm motility," *Human Reproduction*, vol. 18, no. 12, pp. 2582–2589, 2003.

[61] J.-C. Huang, W.-S. A. Wun, J. S. Goldsby, I. C. Wun, D. Noorhasan, and K. K. Wu, "Stimulation of embryo hatching and implantation by prostacyclin and peroxisome proliferator-activated receptor δ activation: implication in IVF," *Human Reproduction*, vol. 22, no. 3, pp. 807–814, 2006.

[62] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[63] O. Braissant and W. Wahli, "Differential expression of peroxisome proliferator-activated receptor-α, -β, and -γ during rat embryonic development," *Endocrinology*, vol. 139, no. 6, pp. 2748–2754, 1998.

[64] B. Löhrke, T. Viergutz, S. K. Shahi, et al., "Detection and functional characterisation of the transcription factor peroxisome proliferator-activated receptor γ in lutein cells," *Journal of Endocrinology*, vol. 159, no. 3, pp. 429–439, 1998.

[65] T. Viergutz, B. Loehrke, R. Poehland, F. Becker, and W. Kanitz, "Relationship between different stages of the corpus luteum and the expression of the peroxisome proliferator-activated receptor γ protein in bovine large lutein cells," *Journal of Reproduction and Fertility*, vol. 118, no. 1, pp. 153–161, 2000.

[66] Y. Cui, K. Miyoshi, E. Claudio, et al., "Loss of the peroxisome proliferation-activated receptor γ (PPARγ) does not affect mammary development and propensity for tumor formation but leads to reduced fertility," *Journal of Biological Chemistry*, vol. 277, no. 20, pp. 17830–17835, 2002.

[67] D. I. Lebovic, M. Kir, and C. L. Casey, "Peroxisome proliferator-activated receptor-γ induces regression of endometrial explants in a rat model of endometriosis," *Fertility and Sterility*, vol. 82, supplement 3, pp. 1008–1013, 2004.

[68] V. Sapin, P. Dollé, C. Hindelang, P. Kastner, and P. Chambon, "Defects of the chorioallantoic placenta in mouse RXRα null fetuses," *Developmental Biology*, vol. 191, no. 1, pp. 29–41, 1997.

[69] T. Shalom-Barak, J. M. Nicholas, Y. Wang, et al., "Peroxisome proliferator-activated receptor γ controls *Muc1* transcription in trophoblasts," *Molecular and Cellular Biology*, vol. 24, no. 24, pp. 10661–10669, 2004.

[70] U. Elchalal, R. G. Humphrey, S. D. Smith, C. Hu, Y. Sadovsky, and D. M. Nelson, "Troglitazone attenuates hypoxia-induced injury in cultured term human trophoblasts," *American Journal of Obstetrics and Gynecology*, vol. 191, no. 6, pp. 2154–2159, 2004.

[71] Y. Wan, A. Saghatelian, L.-W. Chong, C.-L. Zhang, B. F. Cravatt, and R. M. Evans, "Maternal PPARγ protects nursing neonates by suppressing the production of inflammatory milk," *Genes & Development*, vol. 21, no. 15, pp. 1895–1908, 2007.

[72] Y. Jia, C. Qi, Z. Zhang, Y. T. Zhu, S. M. Rao, and Y.-J. Zhu, "Peroxisome proliferator-activated receptor-binding protein null mutation results in defective mammary gland development," *Journal of Biological Chemistry*, vol. 280, no. 11, pp. 10766–10773, 2005.

[73] E. Saez, J. Rosenfeld, A. Livolsi, et al., "PPARγ signaling exacerbates mammary gland tumor development," *Genes & Development*, vol. 18, no. 5, pp. 528–540, 2004.

[74] R. L. Goldenberg, J. C. Hauth, and W. W. Andrews, "Intrauterine infection and preterm delivery," *The New England Journal of Medicine*, vol. 342, no. 20, pp. 1500–1507, 2000.

[75] G. Pascual, A. L. Fong, S. Ogawa, et al., "A SUMOylation-dependent pathway mediates transrepression of inflammatory response genes by PPAR-γ," *Nature*, vol. 437, no. 7059, pp. 759–763, 2005.

[76] L. L. Waite, E. C. Person, Y. Zhou, K.-H. Lim, T. S. Scanlan, and R. N. Taylor, "Placental peroxisome proliferator-activated receptor-γ is up-regulated by pregnancy serum," *Journal of Clinical Endocrinology & Metabolism*, vol. 85, no. 10, pp. 3808–3814, 2000.

[77] M. Lehrke, G. Pascual, C. K. Glass, and M. A. Lazar, "Gaining weight: the keystone symposium on PPAR and LXR," *Genes & Development*, vol. 19, no. 15, pp. 1737–1742, 2005.

[78] K. R. Steffensen, K. Robertson, J.-Å. Gustafsson, and C. Y. Andersen, "Reduced fertility and inability of oocytes to resume meiosis in mice deficient of the *LXR* genes," *Molecular and Cellular Endocrinology*, vol. 256, no. 1-2, pp. 9–16, 2006.

[79] K. Mouzat, M. Prod'homme, D. H. Volle, et al., "Oxysterol nuclear receptor LXRβ regulates cholesterol homeostasis and contractile function in mouse uterus," *Journal of Biological Chemistry*, vol. 282, no. 7, pp. 4693–4701, 2007.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 732303, 7 pages
doi:10.1155/2008/732303

Review Article

The Role of Peroxisome Proliferator-Activated Receptors in the Development and Physiology of Gametes and Preimplantation Embryos

Jaou-Chen Huang

Division of Reproductive Endocrinology and Infertility, Department of Obstetrics, Gynecology and Reproductive Sciences, University of Texas Medical School at Houston, 6431 Fannin Street, Houston, TX 77030, USA

Correspondence should be addressed to Jaou-Chen Huang, jaou-chen.huang@uth.tmc.edu

Received 22 August 2007; Accepted 4 December 2007

Recommended by Pascal Froment

In several species, a family of nuclear receptors, the peroxisome proliferator-activated receptors (PPARs) composed of three isotypes, is expressed in somatic cells and germ cells of the ovary as well as the testis. Invalidation of these receptors in mice or stimulation of these receptors in vivo or in vitro showed that each receptor has physiological roles in the gamete maturation or the embryo development. In addition, synthetic PPARγ ligands are recently used to induce ovulation in women with polycystic ovary disease. These results reveal the positive actions of PPAR in reproduction. On the other hand, xenobiotics molecules (in herbicides, plasticizers, or components of personal care products), capable of activating PPAR, may disrupt normal PPAR functions in the ovary or the testis and have consequences on the quality of the gametes and the embryos. Despite the recent data obtained on the biological actions of PPARs in reproduction, relatively little is known about PPARs in gametes and embryos. This review summarizes the current knowledge on the expression and the function of PPARs as well as their partners, retinoid X receptors (RXRs), in germ cells and preimplantation embryos. The effects of natural and synthetic PPAR ligands will also be discussed from the perspectives of reproductive toxicology and assisted reproductive technology.

1. INTRODUCTION

Peroxisomes are organelles in eukaryotes that remove toxic substances and break down fatty acid. Peroxisome proliferator-activated receptor α (PPARα) was discovered during the search for a compound that increases the proliferation of peroxisomes in mouse liver cells [1]. Subsequently, two additional isotypes, PPARβ (also known as PPARδ) and PPARγ, were discovered. The three PPARs are encoded by different genes. Variants arising from alternative splicing and usage of different promoters have been reported in all three PPARs [2]. Together they form a subfamily within the steroid receptor superfamily. To date, PPARs have been identified in many species, including *Xenopus*, sea squirt, zebrafish, *Aedes aegypti* (yellow fever mosquito), *Anopheles gambiae* (a species complex which contains six vectors of malaria), mouse, rat, hamster, and human (http://www.ensembl.org/index.html).

Since their discovery, a great deal has been learned about PPARα, PPARγ, and, to a less extent, PPARβ/δ. The knowledge has been applied to clinical practice: synthetic PPARα ligands (fibrates) and PPARγ ligands (thiazolidinediones TZD), respectively, are widely used to treat lipid and glucose disorders. In contrast, the use of PPARs to enhance fertility is constrained by our relative meager knowledge regarding PPARs and reproduction. PPARγ activators have recently been used to induce ovulation in women with polycystic ovary disease, a condition of ovulation dysfunction associated with insulin resistance. This review will focus on the roles of PPARs in the development and physiology of gametes and preimplantation embryos. Also included in the discussion are potential impacts of natural or synthetic PPAR ligand on reproduction and the promising benefits of synthetic PPAR ligands in enhancing the success of assisted reproductive technology.

2. PPARs AND RXRs

PPARs, similar to steroid and thyroid hormone receptors, are ligand-activated nuclear transcription factors. Unlike steroid

and thyroid hormone receptors, PPARs were discovered before their functions were fully understood. Over the years, tissue distribution and synthetic ligands, which bind to specific PPAR, helped to elucidate the biological functions of PPARs.

PPARs form heterodimers with another nuclear receptor, retinoid X receptor (RXR). This interaction occurs in the presence and absence of PPAR ligand. The PPAR-RXR complex recruits other cofactors before binding to PPAR responsive element (PPRE) at the promoter regions of PPAR-responsive genes. Besides PPARs, RXR also forms heterodimers with other nuclear receptors. RXR has three isotypes: RXRα, RXRβ, and RXRγ, all of which are activated by 9-*cis*-retinoic acid (but not by all-*trans* retinoic acid) [3]. The 9-*cis*-retinoic acid was originally considered as the endogenous ligand for RXRs in vivo; but recent reports [4, 5] cast considerable doubt that it is the case. Although RXRs exist as three isotypes, they do not confer different functions to PPAR-RXR complexes. The PPAR-RXR complexes are activated by either PPAR or RXR ligand, but simultaneous binding by both ligands elicits more potent activities [6]. A unique feature of PPARβ/δ, not seen in PPARα or PPARγ, is its ability to repress the transcriptional activities of PPARα and PPARγ. This activity is mediated by corepressors recruited by PPARβ/δ [7].

The DNA sequence of PPRE is typically of a direct repeat 1 (DR1) nuclear receptor in that the PPRE DNA sequence consists of two repeats of AGGTCA separated by one nucleotide (AGGTCA N AGGTCA). Detailed analyses of native PPREs show that the consensus PPRE sequence is 5′-AACTAGGNCA A AGGTCA-3′ [6]. The extended 5′ half site, the one imperfect DR1 core, and the adenine as the spacing nucleotide may confer additional selectivity to the binding of PPAR-RXR complex.

3. DISTRIBUTION AND BIOLOGICAL FUNCTIONS OF PPAR

The functions of PPARs can be extrapolated from tissue(s) expressing the specific PPAR isotype or from the functions of genes regulated by specific PPAR. PPARα is expressed most abundantly in brown adipose tissue and liver, followed by the kidney, heart, and skeletal muscle. PPARγ is mainly expressed in adipose tissue and, to a less extent, in the colon, the immune system, and the retina. Both PPARα and PPARγ responsive genes are involved in lipid homeostasis. Therefore, it is not surprising that the main functions of PPARα and PPARγ are in glucose and lipid homeostasis [6, 8].

On the other hand, the ubiquitous distribution of PPARβ/δ (although gut, kidney, and heart express higher levels than other tissues) makes it difficult to associate PPARβ/δ with specific biological function [8]. The multiple functions of PPARβ/δ are revealed by the diverse genes regulated by PPARβ/δ, such as ILK [9], 11 ß hydroxysteroid dehydrogenase II [10], PTEN [9], and 14-3-3ε [11]. It is worth noting that 14-3-3ε functions as a protein chaperone. Therefore, PPARβ/δ is indirectly associated with even more diverse range of functions. Indeed, PPARβ/δ has been implicated in embryo implantation [12], intestinal adenoma [13], colon cancer [14], skin wound healing [15], hair follicle development [16], and cytoprotection [11].

4. PPAR LIGANDS

Natural and synthetic PPAR ligands relevant to this review are listed below. More extensive lists are available in the literature [6, 17].

Unsaturated fatty acids are ligands to all PPARs, with PPARα exhibiting the highest affinity; saturated fatty acids, on the other hand, are not effective PPAR ligands. Eicosanoids derived from arachidonic acid form a unique group of fatty acids that bind to PPARs. They include leukotrienes, hydroxyeicosatetraenoic acids (HETEs) (both are formed via the lipoxygenase pathway), and prostaglandins (PGs) (formed via the cyclooxygenase pathway). Leukotriene B4 and 8(S)-HETE are PPARα ligand; and 15-deoxy-Δ12, 14-PGJ_2 (15d-PGJ_2, a PGD_2 derivative) is a PPARγ ligand. Synthetic PPARα (fibrates) and PPARγ (TZD) ligands are used to lower blood lipid and glucose, respectively. Prostacyclin (PGI_2) is a natural PPARβ/δ ligand, indeed the uterine PGI_2 generated by cyclooxygenase-2 (COX-2) mediates the implantation of embryos via PPARβ/δ [12]. Synthetic PGI_2 analogs, such as iloprost and carbaprostacyclin, may function as PGI_2 receptor agonists or PPARβ/δ ligands. Although iloprost is used as a PGI_2 receptor agonist to treat pulmonary hypertension and peripheral vascular diseases, no PGI_2 analog has been used as a PPARβ/δ ligand clinically. A recent report indicates that retinoic acid, in cells with high fatty acid binding protein 5 to retinoic acid binding protein-II ratio, may function as a natural PPARβ/δ ligand [18]. This finding may have evolutional or developmental significance in germ cell maturation, gamete function, or embryo development. PGI_2 and retinoic acid may provide functional redundancy to ensure PPARβ/δ activation or they may compliment each other to activate PPARβ/δ in a developmental stage-dependent manner based on the ratio of the two binding proteins.

5. PPAR LIGANDS IN THE REPRODUCTIVE TRACT

Zygotes remain in the oviduct after fertilization and develop to morula or early blastocyst stage embryos before entering into the uterus. It is generally accepted that, compared with cultured embryos (derived from fertilized eggs in vitro or flushed from oviducts at earlier developmental stage), in vivo embryos develop better and have less cell death because oviducts protect the embryos and promote their development [19]. The unique environment provided by the oviduct includes oviduct-derived soluble factors and embryo-derived autocrine factors. Both oviducts and embryos are sources of PPAR ligand(s).

Earlier studies show that the oviduct produces abundant PGE_2 and $PGF_2\alpha$, which regulate its motility. We serendipitously discovered that human [20] and mouse [21] oviducts produce other eicosanoids that activate PPARs. PGI_2 (a PPARβ/δ ligand) is the most abundant product, PGD_2 (whose derivative, 15d-PGJ_2, is a PPARγ ligand), and other products derived from the lipoxygenase pathway are

also produced in substantial amounts. PGI_2 synthesis by mouse oviducts is synchronized with estrus cycles (and, thus, the development of preimplantation embryos). Peak PGI_2 synthetic capacity coincides with the window of receptivity, that is, between the eight-cell and morula stages [21, 22].

Recent reports indicate that human [23] and mouse [24, 25] preimplantation embryos express COX isoenzymes and synthesize eicosanoids. PGI_2 is the most abundant metabolite when radio-labeled arachidonic acid is incubated with blastocyst-stage mouse embryos. Other eicosanoids, such as HETEs and PGD_2 are also produced by mouse blastocysts [24].

6. RXR IN GAMETES AND PREIMPLANTATION EMBRYOS

Gametes and preimplantation embryos express RXRs. Whereas RXRγ-null mice are normal [26], RXRα- [27] and RXRβ-null mice [28] have distinctive phenotypes. Gene knockout studies show that spermatogenesis requires RXRβ. Similarly, oocyte development may be modulated by RXR, which is expressed in both granulosa-cumulus cells and oocytes. Finally, the quality of embryo development may be associated with RXR expression.

6.1. RXR in gametes

RXRα and RXRβ are expressed in human cumulus granulosa cells [29] and bovine oocytes [30]. Although the initial reports on RXRα [27] and RXRβ [28] null mice did not include a description of female reproduction (such as follicular development and ovulation), the localization of RXRα and β in the ovary supports their roles in follicular maturation and oocyte function. RXR may regulate oocyte development directly (via modulating steroidogenesis in the granulosa cells) or indirectly (by affecting oocyte gene transcription) [31]. It is likely that female mice with targeted RXR deletion may suffer subfertility.

The male sterility observed in RXRβ-null mice [28] underscores the essential role of RXR (and its functional partner) in spermatogenesis. In mouse testes, retinoic acid receptors (RARs) and RXR are expressed in well-defined cell populations: RARα and RXRβ in Sertoli cells, RARβ, RXRα, and RXRγ in steps 7 and 8 spermatids, and RARγ in spermatogonia. Mouse spermatocytes, however, do not express RARs [32]. Although RARβ, RXRα, and RXRγ are coexpressed in step 7 and 8 spermatids, RARβ may not functionally couple with either RXRα or RXRγ, because RARβ-, RXRγ-, and RARβ/RXRγ-null mice do not display reproductive defects [32]. On the other hand, RXRβ and RARα may form heterodimer and control spermiation in vivo because both are coexpressed in Sertoli cells and invalidation of either gene in mice leads to similar phenotype [32]. RXRβ-null males are sterile due to oligoasthenoteratozoospermia caused by failed spermatid release (from the germinal epithelium) and abnormal sperm acrosomes and tails [27]. In Sertoli cells, the function of RXRβ (coupled with RARα) may involve lipid metabolism or transport, because they progressively accumulate lipids (which are unsaturated triglycerides) in RXRβ-null mice. In older RXRβ-null males, germ cells degenerate completely and seminiferous tubules are filled with lipid vacuoles [27]. RARα homozygous mutant [33] and mice with targeted RARα ablation in Sertoli cells [34] display similar phenotype. Both have testicular degeneration, failed spermiation, epithelial vacuolation, germ cell desquamation, and apoptosis [34]. Although there is no report concerning RXR expression in spermatozoa, it can be inferred that human sperm express RXR because human sperm express PPARγ (which forms functional complex with RXR) and PPARγ ligand enhances their activities [35].

6.2. RXR in preimplantation embryos

The development of preimplantation embryos was not described in the initial reports describing RXRα- [27] and RXRβ- [28] null mice. However, available information in the literature shows that preimplantation embryos express RXRs. Transcripts of RXRα, RXRβ, and RXRγ are expressed in zebrafish embryos at 1.5 hour postfertilization [36]. RXRα, RXRβ, and $RALDH_2$ (one of the two enzymes oxidizing retinol to retinoic acid) are detected in all stages of preimplantation bovine embryos, including blastocysts which express RXRβ protein in the inner cell mass and the trophectoderm [30]. RXRα, β, and γ transcripts in preimplantation bovine embryos are likely of maternal origin because eight-cell stage and earlier embryos have significantly higher RXR levels than later stage embryos [37]. Furthermore, RXRs may be essential for optimal embryo development because "good-quality" embryos express significantly higher levels of RXR transcripts than "bad-quality" embryos [37]. It can be summarized that RXR expression in preimplantation embryos described above is corroborated by the expression of its partner, PPAR (discussed later). Furthermore, RXR (partners with PPAR or RAR) is crucial to normal embryo development because (1) early stage embryos contain high levels of maternal RXR mRNA, and (2) "good-quality" embryos express higher RXR mRNA levels.

7. PPAR IN GAMETES AND PREIMPLANTATION EMBRYOS

Compared with their role in postimplantation embryo development, the roles of PPARs in fertilization, implantation, and embryo development are less well defined. Available information does suggest that gametes and preimplantation embryos express functional PPARs and that PPAR activation optimizes their functions.

7.1. PPAR and gametes

All three PPAR isotypes are expressed in somatic and germ cells of the testis. In rat, PPARα and β/δ are expressed in Leydig cells and Sertoli cells [38]. In human, PPARγ1 message is detected in the testis [39]. In mouse, both PPARα and γ are expressed in Sertoli cells [40], and PPARβ/δ is expressed in spermatids and spermatocytes [41]. The expression of PPARβ/δ in mouse spermatids and spermatocytes is further supported by the expression of *Ssm*, a novel PPARβ/δ

target gene, in mouse testis [42]. The functionality of PPARα in Sertoli cells is confirmed by its nuclear translocation in response to a selective PPARα ligand, Wy-14,643 [40]. These findings suggest that PPARs (in Sertoli cells and Leydig cells) provide an environment for spermatogenesis and may be directly involved in germ cell maturation. PPAR may regulate germ cell maturation in a stage-dependent fashion. In zebrafish, PPARγ is expressed in spermatogonia but not in spermatocytes [43].

PPAR ligand affects spermatogenesis and sperm activities. Di(*n*-butyl) phthalate, a PPAR activator, modulates the expression of genes related to spermatogenesis and steroidogenesis and causes testicular atrophy in rats [44]. In contrast, the capacitation, acrosome reaction, and motility of ejaculated human sperm are enhanced by a treatment with rosiglitazone (a synthetic PPARγ ligand) or 15d-PGJ2 (a natural PPARγ ligand) [35]. Since germ cells express all three PPAR isotypes, the expression and function of two other PPAR isotypes, PPARα and β/δ, in mature spermatozoa warrant further investigation.

In several species including rat, all three PPAR isotypes are detected in the ovary [2]. PPARγ, which has been studied more extensively than the other two isotypes, is detected in the mouse, rat, pig, sheep, cow, and human ovary. PPARγ is expressed strongly in the granulosa cells of rat [2], mouse [41], and sheep [45], as well as in oocytes from cattle [30], zebrafish [43], *Xenopus* [46], and human [47]. PPARγ is detected in different classes of follicles (primary/secondary to preovulatory follicles) and its expression increases with the development of follicles. After the LH surge, PPARγ mRNA expression is downregulated [2]. Activation of PPARγ by natural and synthetic ligands in the granulosa cells appears to regulate the synthesis of steroid hormones. Thus, PPARγ may be indirectly involved in oocyte maturation via the granulosa cells. Indeed, disruption of PPARγ gene in the ovary using *cre/loxP* technology led to female subfertility [48]. On the other hand, PPARs may be directly involved in oocyte maturation. Indeed, it has been reported that rosiglitazone, a synthetic PPARγ ligand, at 100 μM stimulates AMP-activated protein kinase (AMPK) and enhances the meiotic resumption of mouse oocytes [42].

7.2. PPAR and preimplantation embryos

Preimplantation bovine and mouse embryos express PPARγ and PPARβ/δ, respectively. Beginning at two-cell stage and throughout the preimplantation period, bovine embryos express PPARγ. Blastocyst stage bovine embryos express PPARγ in the inner cell mass and the trophectoderm [30]. Mouse embryos express PPARβ/δ detectable by immunohistochemistry at two-cell stage [25] or eight-cell stage [22] and throughout the preimplantation period. Mouse blastocysts also express PPARβ/δ in the inner cell mass and the trophectoderm [22].

Although preimplantation embryo development and implantation were not specifically examined in the initial report regarding PPARβ/δ-null mouse, the report provides a hint of the impacts of PPARβ/δ deficiency [49]. The genotypic distribution of embryos on gestation day 9.5 shows that PPAR$\beta/\delta -/-$ embryos are underrepresented: PPAR$\beta/\delta -/-$ embryos represent 16% (3/19) and 38% (3/8) of embryos from PPAR$\beta/\delta +/-$ x PPAR$\beta/\delta +/-$ and PPAR$\beta/\delta -/-$ x PPAR$\beta/\delta +/-$ mating, respectively. This represents a 36% (i.e., 25% versus 16%) and a 24% (i.e., 50% versus 38%) deviation from the expected Mendelian frequency. Loss of PPAR$\beta/\delta -/-$ embryos prior to gestation day 9.5 may occur at any stage including ovulation, fertilization, preimplantation period, implantation, and postimplantation period up to gestation day 9.5. The results of our study show that PPARβ/δ ablation adversely affects preimplantation embryo development and, consequently, implantation [22]. Compared with wild-type embryos, PPAR$\beta/\delta -/-$ embryos show developmental delay as early as 48 hours after two-cell stage embryos are harvested. The gap widens in the subsequent 48 hours. At 96 hours after the harvest of two-cell embryos, 100% of wild-type embryos have reached or passed the blastocyst stage (versus 65% PPAR$\beta/\delta -/-$ embryos), and 85% of wild-type embryos have undergone hatching or hatched completely (versus 28% PPAR$\beta/\delta -/-$ embryos). Consequently, PPAR$\beta/\delta -/-$ embryos implant less effectively than wild-type embryos (28% versus 44%). We also found that PPAR$\beta/\delta -/-$ embryos have decreased embryonic cell proliferation compared with that observed in wild-type embryos. These results suggest that PPARβ/δ activation via endogenous PPARβ/δ ligand, such as PGI_2 [24] and/or retinoic acid [18], confers the "basal" momentum (including cell proliferation and possibly other functions) to preimplantation embryos and propels them through various stages of development.

In addition to providing a "basal" momentum of embryo development via endogenous PPARβ/δ ligand, PPARβ/δ activation by synthetic ligand further enhances the development and the implantation of cultured embryos. Both L-165041 (a synthetic PPARβ/δ ligand) and iloprost (a stable PGI_2 analog) enhance complete embryo hatching in a concentration-dependent manner [22, 50, 51]. Embryos preconditioned with L-165041 or iloprost show higher implantation rates when transferred to gestational carriers [22, 52]. These results suggest that cultured embryos do not reach their full developmental potential due to insufficient endogenous PPARβ/δ ligands or lack of exogenous PPARβ/δ ligands normally provided by the oviduct. Embryos exposed to PPARβ/δ ligand have increased embryonic cell proliferation compared with controlled embryos [22].

8. PPAR AND REPRODUCTIVE TOXICOLOGY

PPAR activators are found in herbicides, industrial plasticizers (for a brief review see [53]), and personal care products such as hair spray and solvent for perfumes [54]. Di(*n*-butyl) phthalate, a PPARγ activator found in plasticizers and personal care products, may cause male infertility by altering hormones involved in steroidogenesis and spermatogenesis [44]. Other potential PPAR activators posing reproductive toxicology concerns are pharmaceutical agents used to lower lipids and blood glucose. Rosiglitazone (a TZD for diabetes) may activate PPARγ ligand and enhance sperm activities in men [35] or, depending on its concentration, may enhance

meiosis resumption of oocytes or induce oocyte degeneration in women [55]. Nonsteroidal anti-inflammatory drugs, such as Motrin, which blocks PG synthesis, pose reproductive hazards through a different mechanism. Decreased PG (such as PGI_2) production may adversely affect embryo development and implantation.

On the other hand, PPARs may be exploited to enhance the success of assisted reproductive technology. The fertilization potentials of human sperm in in vitro fertilization (IVF) or other assisted reproductive technologies, such as artificial insemination, may be enhanced by incubating sperm with synthetic PPARγ ligands. The development and implantation of IVF embryos may be augmented by supplementing culture media with PGI_2 analogs, synthetic PPARβ/δ ligand, or retinoic acid. However, potential long-term adverse effects are unknown. Large-scale clinical trials of sufficient power are needed to validate the benefits and to assess the harms.

9. CONCLUSION

The literature on PPARs in gametes and preimplantation embryos is relatively limited. Nonetheless, the consensus is that PPAR serves to optimize gamete function and embryo development. Further studies are needed to shed more light on the physiological roles of PPARs in reproduction. The knowledge gained will help us avoid potential reproductive hazards and augment the success of assisted reproductive technologies.

REFERENCES

[1] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[2] C. M. Komar, "Peroxisome proliferator-activated receptors (PPARs) and ovarian function—implications for regulating steroidogenesis, differentiation, and tissue remodeling," *Reprod Biol Endocrinol*, vol. 3, p. 41, 2005.

[3] D. J. Mangelsdorf, C. Thummel, M. Beato, et al., "The nuclear receptor superfamily: the second decade," *Cell*, vol. 83, no. 6, pp. 835–839, 1995.

[4] F. A. Mic, A. Molotkov, D. M. Benbrook, and G. Duester, "Retinoid activation of retinoic acid receptor but not retinoid X receptor is sufficient to rescue lethal defect in retinoic acid synthesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 12, pp. 7135–7140, 2003.

[5] G. Wolf, "Is 9-*cis*-retinoic acid the endogenous ligand for the retinoic acid-X receptor?" *Nutrition Reviews*, vol. 64, no. 12, pp. 532–538, 2006.

[6] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[7] Y. Shi, M. Hon, and R. M. Evans, "The peroxisome proliferator-activated receptor δ, an integrator of transcriptional repression and nuclear receptor signaling," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 5, pp. 2613–2618, 2002.

[8] S. Kersten, B. Desvergne, and W. Wahli, "Roles of PPARS in health and disease," *Nature*, vol. 405, no. 6785, pp. 421–424, 2000.

[9] N. Di-Poi, N. S. Tan, L. Michalik, W. Wahli, and B. Desvergne, "Antiapoptotic role of PPARβ in keratinocytes via transcriptional control of the Akt1 signaling pathway," *Molecular Cell*, vol. 10, no. 4, pp. 721–733, 2002.

[10] L. Julan, H. Guan, J. P. van Beek, and K. Yang, "Peroxisome proliferator-activated receptor δ suppresses 11β-hydroxysteroid dehydrogenase type 2 gene expression in human placental trophoblast cells," *Endocrinology*, vol. 146, no. 3, pp. 1482–1490, 2005.

[11] J.-Y. Liou, S. Lee, D. Ghelani, N. Matijevic-Aleksic, and K. K. Wu, "Protection of endothelial survival by peroxisome proliferator-activated receptor-δ mediated 14-3-3 upregulation," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 7, pp. 1481–1487, 2006.

[12] H. Lim, R. A. Gupta, W. G. Ma, et al., "Cyclo-oxygenase-2-derived prostacyclin mediates embryo implantation in the mouse via PPARδ," *Genes & development*, vol. 13, no. 12, pp. 1561–1574, 1999.

[13] R. A. Gupta, D. Wang, S. Katkuri, H. Wang, S. K. Dey, and R. N. DuBois, "Activation of nuclear hormone receptor peroxisome proliferator-activated receptor-δ accelerates intestinal adenoma growth," *Nature Medicine*, vol. 10, pp. 245–247, 2004.

[14] R. A. Gupta, J. Tan, W. F. Krause, et al., "Prostacyclin-mediated activation of peroxisome proliferator-activated receptor δ in colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 97, no. 24, pp. 13275–13280, 2000.

[15] N. Di-Poi, L. Michalik, N. S. Tan, B. Desvergne, and W. Wahli, "The anti-apoptotic role of PPARβ contributes to efficient skin wound healing," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 85, no. 2–5, pp. 257–265, 2003.

[16] N. Di-Poi, C. Y. Ng, N. S. Tan, et al., "Epithelium-mesenchyme interactions control the activity of peroxisome proliferator-activated receptor β/δ during hair follicle development," *Molecular and Cellular Biology*, vol. 25, no. 5, pp. 1696–1712, 2005.

[17] B. M. Forman, J. Chen, and R. M. Evans, "Hypolipidemic drugs, polyunsaturated fatty acids, and eicosanoids are ligands for peroxisome proliferator-activated receptors α and δ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, pp. 4312–4317, 1997.

[18] T. T. Schug, D. C. Berry, N. S. Shaw, S. N. Travis, and N. Noy, "Opposing effects of retinoic acid on cell growth result from alternate activation of two different nuclear receptors," *Cell*, vol. 129, no. 4, pp. 723–733, 2007.

[19] K. Hardy and S. Spanos, "Growth factor expression and function in the human and mouse preimplantation embryo," *Journal of Endocrinology*, vol. 172, no. 2, pp. 221–236, 2002.

[20] J.-C. Huang, F. Arbab, K. J. Tumbusch, J. S. Goldsby, N. Matijevic-Aleksic, and K. K. Wu, "Human Fallopian tubes express prostacyclin (PGI) synthase and cyclooxygenases and synthesize abundant PGI," *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 9, pp. 4361–4368, 2002.

[21] J.-C. Huang, J. S. Goldsby, F. Arbab, Z. Melhem, N. Aleksic, and K. K. Wu, "Oviduct prostacyclin functions as a paracrine factor to augment the development of embryos," *Human Reproduction*, vol. 19, no. 12, pp. 2907–2912, 2004.

[22] J.-C. Huang, W. S. Wun, J. S. Goldsby, I. C. Wun, D. Noorhasan, and K. K. Wu, "Stimulation of embryo hatching and implantation by prostacyclin and peroxisome proliferator-activated receptor δ activation: implication in IVF," *Human Reproduction*, vol. 22, no. 3, pp. 807–814, 2007.

[23] H. Wang, Y. Wen, S. Mooney, B. Behr, and M. L. Polan, "Phospholipase A_2 and cyclooxygenase gene expression in human preimplantation embryos," *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 6, pp. 2629–2634, 2002.

[24] J.-C. Huang, W.-S. A. Wun, J. S. Goldsby, N. Matijevic-Aleksic, and K. K. Wu, "Cyclooxygenase-2-derived endogenous prostacyclin enhances mouse embryo hatching," *Human Reproduction*, vol. 19, no. 12, pp. 2900–2906, 2004.

[25] P. L. Pakrasi and A. K. Jain, "Evaluation of cyclooxygenase 2 derived endogenous prostacyclin in mouse preimplantation embryo development in vitro," *Life Sciences*, vol. 80, no. 16, pp. 1503–1507, 2007.

[26] W. Krezel, V. Dupe, M Mark, A. Dierich, P. Kastner, and P. Chambon, "RXR gamma null mice are apparently normal and compound RXR alpha +/-/RXR beta -/-/RXR gamma -/- mutant mice are viable," *Proceedings of the National Academy of Sciences of the United states of America*, vol. 93, no. 17, pp. 9010–9014, 1996.

[27] P. Kastner, J. M. Grondona, M. Mark, et al., "Genetic analysis of RXRα developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis," *Cell*, vol. 78, no. 6, pp. 987–1003, 1994.

[28] P. Kastner, M. Mark, M. Leid, et al., "Abnormal spermatogenesis in RXR β mutant mice," *Genes & development*, vol. 10, pp. 80–92, 1996.

[29] M. Mohan, N. R. Thirumalapura, and J. Malayer, "Bovine cumulus-granulosa cells contain biologically active retinoid receptors that can respond to retinoic acid," *Reproductive Biology and Endocrinology*, vol. 1, p. 104, 2003.

[30] M. Mohan, J. R. Malayer, R. D. Geisert, and G. L. Morgan, "Expression patterns of retinoid X receptors, retinaldehyde dehydrogenase, and peroxisome proliferator activated receptor γ in bovine preattachment embryos," *Biology of Reproduction*, vol. 66, no. 3, pp. 692–700, 2002.

[31] S. Minucci, J. Wong, J. C. G. Blanco, Y.-B. Shi, A. P. Wolffe, and K. Ozato, "Retinoid receptor-induced alteration of the chromatin assembled on a ligand-responsive promoter in *Xenopus* oocytes," *Molecular Endocrinology*, vol. 12, no. 3, pp. 315–324, 1998.

[32] N. Vernet, C. Dennefeld, C. Rochette-Egly, et al., "Retinoic acid metabolism and signaling pathways in the adult and developing mouse testis," *Endocrinology*, vol. 147, no. 1, pp. 96–110, 2006.

[33] T. Lufkin, D. Lohnes, M. Mark, et al., "High postnatal lethality and testis degeneration in retinoic acid receptor α mutant mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 90, pp. 7225–7229, 1993.

[34] N. Vernet, C. Dennefeld, F. Guillou, P. Chambon, N. B. Ghyselinck, and M. Mark, "Prepubertal testis development relies on retinoic acid but not rexinoid receptors in Sertoli cells," *EMBO Journal*, vol. 25, no. 24, pp. 5816–5825, 2006.

[35] S. Aquila, D. Bonofiglio, M. Gentile, et al., "Peroxisome proliferator-activated receptor (PPAR)γ is expressed by human spermatozoa: its potential role on the sperm physiology," *Journal of Cellular Physiology*, vol. 209, no. 3, pp. 977–986, 2006.

[36] A. Tallafuss, L. A. Hale, Y.-L. Yan, L. Dudley, J. S. Eisen, and J. H. Postlethwait, "Characterization of retinoid-X receptor genes rxra, rxrba, rxrbb and rxrg during zebrafish development," *Gene Expression Patterns*, vol. 6, no. 5, pp. 556–565, 2006.

[37] S. Mamo, S. Ponsuksili, K. Wimmers, M. Gilles, and K. Schellander, "Expression of retinoid X receptor transcripts and their significance for developmental competence in in vitro-produced pre-implantation-stage bovine embryos," *Reproduction in Domestic Animals*, vol. 40, no. 2, pp. 177–183, 2005.

[38] O. Braissant, F. Foufelle, C. Scotto, M. Dauca, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, pp. 354–366, 1996.

[39] A. Elbrecht, Y. Chen, C. A. Cullinan, et al., "Molecular cloning, expression and characterization of human peroxisome proliferator activated receptors γ1 and γ2," *Biochemical and Biophysical Research Communications*, vol. 224, no. 2, pp. 431–437, 1996.

[40] N. Bhattacharya, J. M. Dufour, M. N. Vo, J. Okita, R. Okita, and K. H. Kim, "Differential effects of phthalates on the testis and the liver," *Biology of Reproduction*, vol. 72, pp. 745–754, 2005.

[41] H. Higashiyama, A. N. Billin, Y. Okamoto, M. Kinoshita, and S. Asano, "Expression profiling of peroxisome proliferator-activated receptor-δ (PPAR-δ) in mouse tissues using tissue microarray," *Histochemistry and Cell Biology*, vol. 127, no. 5, pp. 485–494, 2007.

[42] K. Han, H. Song, I. Moon, et al., "Utilization of DR1 as true RARE in regulating the Ssm, a novel retinoic acid-target gene in the mouse testis," *Journal of Endocrinology*, vol. 192, pp. 539–551, 2007.

[43] A. Ibabe, M. Grabenbauer, E. Baumgart, H. D. Fahimi, and M. P. Cajaraville, "Expression of peroxisome proliferator-activated receptors in zebrafish (Danio rerio)," *Histochemistry and Cell Biology*, vol. 118, no. 3, pp. 231–239, 2002.

[44] J. Y. Ryu, B. M. Lee, S. Kacew, and H. S. Kim, "Identification of differentially expressed genes in the testis of Sprague-Dawley rats treated with di(*n*-butyl) phthalate," *Toxicology*, vol. 234, no. 1-2, pp. 103–112, 2007.

[45] P. Froment, S. Fabre, J. Dupont, et al., "Expression and functional role of peroxisome proliferator-activated receptor-γ in ovarian folliculogenesis in the sheep," *Biology of Reproduction*, vol. 69, pp. 1665–1674, 2003.

[46] C. Dreyer and H. Ellinger-Ziegelbauer, "Retinoic acid receptors and nuclear orphan receptors in the development of Xenopus laevis," *International Journal of Developmental Biology*, vol. 40, no. 1, pp. 255–262, 1996.

[47] J. R. Wood, D. A. Dumesic, D. H. Abbott, and J. F. Strauss III, "Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis," *Journal of Clinical Endocrinology & Metabolism*, vol. 92, no. 2, pp. 705–713, 2007.

[48] Y. Cui, K. Miyoshi, E. Claudio, et al., "Loss of the peroxisome proliferation-activated receptor γ (PPARγ) does not affect mammary development and propensity for tumor formation but leads to reduced fertility," *Journal of Biological Chemistry*, vol. 277, no. 20, pp. 17830–17835, 2002.

[49] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[50] J.-C. Huang, W.-S. A. Wun, J. S. Goldsby, I. C. Wun, S. M. Falconi, and K. K. Wu, "Prostacyclin enhances embryo hatching but not sperm motility," *Human Reproduction*, vol. 18, no. 12, pp. 2582–2589, 2003.

[51] C.-H. Liu, M.-S. Lee, C.-H. Hsieh, C.-C. Huang, H.-M. Tsao, and Y.-S. Hsieh, "Prostacyclin enhances mouse embryo development and hatching but not increased embryonic cell

number and volume," *Fertility and Sterility*, vol. 86, no. 4, pp. 1047–1052, 2006.

[52] J.-C. Huang, J. S. Goldsby, and W.-S.A. Wun, "Prostacyclin enhances the implantation and live birth potentials of mouse embryos," *Human Reproduction*, vol. 19, no. 8, pp. 1856–1860, 2004.

[53] E. A. Lock, A. M. Mitchell, and C. R. Elcombe, "Biochemical mechanisms of induction of hepatic peroxisome proliferation," *Annual Review of Pharmacology and Toxicology*, vol. 29, pp. 145–163, 1989.

[54] R. H. McKee, J. H. Butala, R. M. David, and G. Gans, "NTP center for the evaluation of risks to human reproduction reports on phthalates: addressing the data gaps," *Reproductive Toxicology*, vol. 18, no. 1, pp. 1–22, 2004.

[55] J. Chen, E. Hudson, M. M. Chi, et al., "AMPK regulation of mouse oocyte meiotic resumption in vitro," *Developmental Biology*, vol. 291, no. 2, pp. 227–238, 2006.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 243791, 19 pages
doi:10.1155/2008/243791

Review Article

PPAR Gamma: Coordinating Metabolic and Immune Contributions to Female Fertility

Cadence E. Minge, Rebecca L. Robker, and Robert J. Norman

Research Centre for Reproductive Health, School of Paediatrics and Reproductive Health, The University of Adelaide, South Australia 5005, Australia

Correspondence should be addressed to Robert J. Norman, robert.norman@adelaide.edu.au

Received 2 April 2007; Accepted 2 July 2007

Recommended by Pascal Froment

Peroxisome proliferator-activated receptor gamma (PPARG) regulates cellular functions such as adipogenesis and immune cell activation. However, new information has indicated additional roles of PPARG directing the cyclic changes that occur within ovarian tissue of female mammals, including those that facilitate the release of oocytes each estrous cycle. In addition to ovarian PPARG expression and function, many PPARG actions within adipocytes and macrophages have additional direct and indirect implications for ovarian function and female fertility. This encompasses the regulation of lipid uptake and transport, insulin sensitivity, glucose metabolism, and the regulation of inflammatory mediator synthesis and release. This review discusses the developing links between PPARG activity and female reproductive function, and highlights several mechanisms that may facilitate such a relationship.

1. INTRODUCTION

Since its initial identification in the early 1990's, peroxisome proliferator-activated receptor gamma (PPARG) has been primarily recognised as a regulator of cellular functions such as adipogenesis and immune cell activation. However, some recent reviews have discussed additional roles of PPARG directing the cyclic changes that occur within ovarian tissue of female mammals, including those that facilitate the release of oocytes each estrous cycle [1–4]. In addition to ovarian PPARG expression and function, many PPARG actions within adipocytes and macrophages have additional direct and indirect implications for ovarian function and female fertility. For instance, PPARG, through activation by thiazolidinediones (TZDs), is known to regulate the metabolism of lipids, providing both self-regulatory PPARG transcriptional mechanisms, and stimulating an increase in adipogenesis. Whilst the net volume of adipose tissue carried within an individual can influence reproductive potential, genes associated with lipid metabolism are also important for ovarian cells directly. As a result, PPARG has the potential to influence the cellular operations of follicles containing oocytes and, consequently, the health of those oocytes released. Likewise, the PPARG regulation of insulin sensitivity, downstream signalling pathways, and ultimately glucose uptake are likely to be also vitally important for normal ovarian function and overall female fertility.

Similarly, PPARG regulation of macrophage function has been addressed in vitro and within the context of the adipose tissue for many years; but appropriate activity of resident immune cells is also a prerequisite for normal ovarian function, as they are required for tissue remodelling facilitating ovulation, luteinization, and luteolysis [5]. Therefore, not only are adipose/circulating macrophage-sourced inflammatory mediators sensed by ovarian cells, but these mediators, when produced locally by the ovary, may influence the ovarian function in an autocrine fashion.

This review aims to provide evidence for how PPARG-regulated pathways influence the female's ability to produce healthy, developmentally competent oocytes. This is impacted by cellular function operating primarily at the local ovarian level, either directly acting upon the oocyte itself, or influencing the supporting ovarian cells that supply the oocyte with hormonal signals and nutrients. In addition,

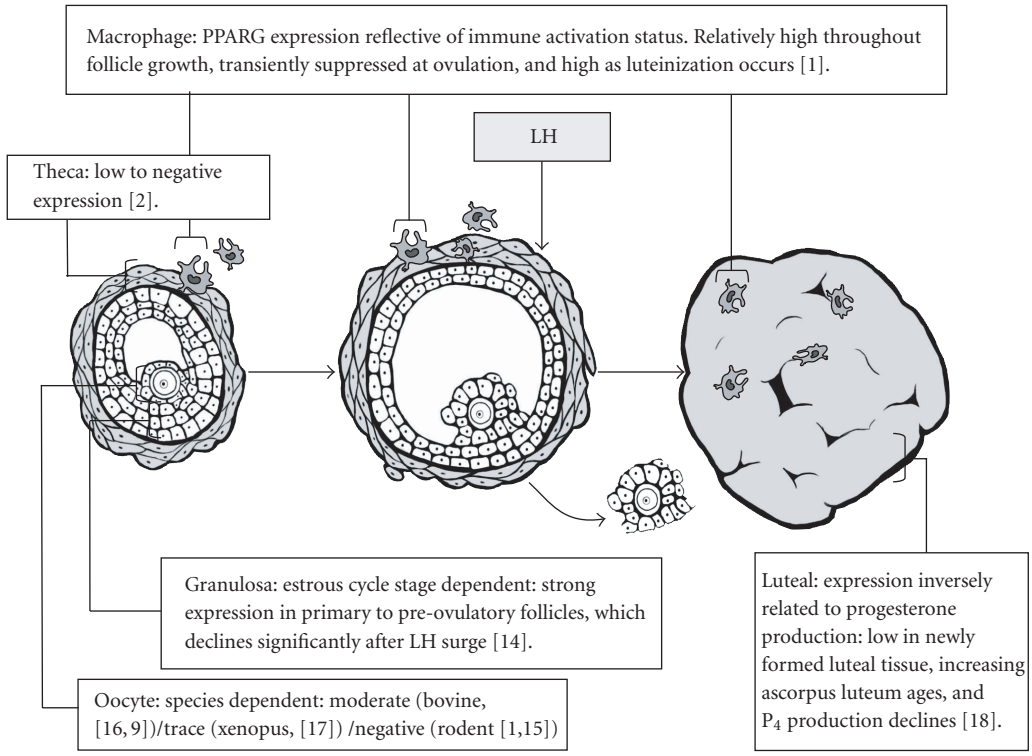


Figure 1: Overview of PPARG expression by specific ovarian cell types, as follicular development progresses from early antral and preovulatory follicle to postovulatory corpus luteum.

signals from extraovarian tissues, in particular adipose tissue and the circulating and/or resident immune cells, also exert powerful influences over the normal function of the ovary.

These concepts of overlapping influence on female fertility are particularly important when we consider conditions of reduced and impaired fertility such as polycystic ovary syndrome (PCOS), as well as reduction of reproductive function associated with excessive bodyweight and insulin resistance. In these situations, profound dysregulation of both metabolic and immune signalling pathways exacerbate ovarian perturbations, which are often successfully treated with administration of PPARG-activating pharmaceuticals.

2. PPARG GENE EXPRESSION

Successful mammalian reproduction requires a female body adequately, but not excessively, nourished, equipped to produce healthy eggs and to supply a growing fetus with sufficient energy. In this way, many tissues within the female body are able to influence the level of fertility. The extent of PPARG expression and its temporal regulation within these tissues can provide an interesting insight into the role of PPARG in female fertility.

2.1. Ovarian PPARG

Within the ovary, processes that are modulated by the PPAR superfamily, particularly PPARG, are among the most critical to normal ovarian function (Figure 1). Steroidogenesis, tissue remodelling, angiogenesis, lipid metabolism, immune cell activation, and production of proinflammatory mediators are all, to some extent, controlled by the presence and activity of the PPAR nuclear receptors. All three PPAR isotypes have been identified in the ovary of many species including the rat [2, 6], mouse [7], pig [8], sheep [9], cow [10, 11], and human [12, 13]. Localisation of these nuclear receptors has been established by both in situ hybridisation and immunohistochemistry [6]. Transcripts for PPAR alpha (PPARA) have been identified in immune cells and cells in the theca and stroma, whilst PPAR delta (PPARD) is found across all ovarian compartments [2]. Ovarian expression of both PPARA and PPARD is relatively stable across the ovulatory cycle, which suggests these isotypes are likely involved in regulating basal ovarian functions. PPARG is expressed strongly in the granulosa cells (primarily responsible for both estradiol production and the regulation of follicular fluid content), and lessstrongly in the thecalregion (site of androgen precursor production for granulosa estradiol synthesis) and luteal cells (postovulatory progesterone production) in the ovaries of rodents and ruminants [1, 2, 9, 14]. PPARG is detected earlyin folliculogenesis, and in contrast to PPARA and PPARD isotypes, its expression is dynamic, increasing until the large folliclestage [9], followed by downregulation in response to the LH surge [2].

Within the oocyte itself, PPARG expression seems to be dependent upon species, as moderate expression has been reported in ruminants [16], trace levels in Xenopus oocytes [17], and undetectable expression in rodents [1, 18]. It has not yet been investigated within the human oocyte.

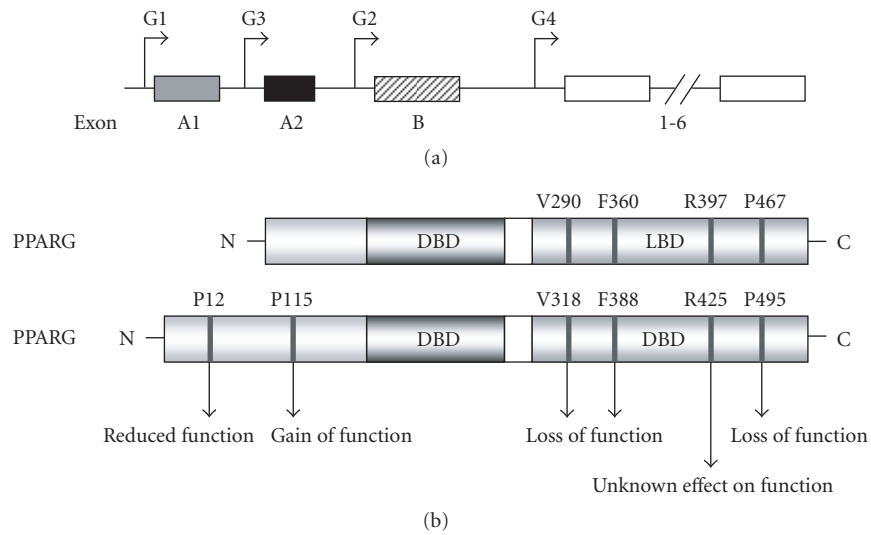


Figure 2: (a) The genomic structure of the 5′ end of the human PPARG gene. Exons 1-6 are common. Exons A1 and A2 are untranslated, and exon B is translated, giving rise to two different proteins corresponding to the G1 or G2 transcripts. (b) The domain structure of PPARG1 and G2 isoforms with the positioning of mutations or polymorphisms resulting in substituted amino acid residues, and altered protein functions. DBD, DNA-binding domain; LBD, Ligand-binding domain. (Figure adapted from Sundvold and Lien[33], Tsai and Maeda [37], and Stumvoll and Häring [38]).

2.2. Extraovarian PPARG

The highest level of mammalian PPARG expression is found within adipose tissue [19, 20], and activation of this adipose PPARG is sufficient [21] and essential to induce adipogenesis [22, 23]. Adiposity is also a key regulator of female fertility, affecting multiple aspects of the reproductive axis in women [24, 25]. Many of the adipocyte-sourced factors that are under PPARG control, such as the production of non-esterified free fatty acids, have widespread effects including ovarian targets [26–28]. In addition, any activation of adipose PPARG that may influence the amount and activity of adipocytes/adipokines can subsequently impact upon reproductive potential [29].

Both the ovary and adipose tissue are comprised of a considerable proportion of immune cells, in particular macrophages. Macrophages recruited into tissues are an important source of many inflammatory mediators that have functions both locally and systemically. Within the ovary, macrophage contribution to the pool of functional PPARG has been assessed [1]. TZD treatment has also been found to affect adipose-recruited macrophages, by increasing the rate of apoptosis, providing a subsequent reduction in the number of proinflammatory cytokine-producing cells [30]. Improvements to the chronically inflamed profile of women with PCOS may well go some way in explaining the beneficial systemic effects of PPARG activation in such patients (see Sections 3.2 and 4.4).

2.3. Mutations in PPARG negatively influence female fertility

The PPARG gene contains 9 exons, and spans more than 100 kb [31] (Figure 2(a)). There are at least 4 isoforms of PPARG, resulting from the use of different initiator methionines [31–33], which are believed to be involved in regulated gene expression in specific cells and tissues. PPARG1, expressed utilizing the untranslated exons A1 and A2, is 477 amino acids long, and is expressed at low levels in many tissues [34]. PPARG2 contains the translated exon B, and as a result is 28 amino acids longer than PPARG1 [35]. This isotype is expressed selectively in white adipose tissue, colonic epithelium, and macrophages [36]. PPARG3, which contains only exon A2, is found only in the large intestine and macrophages [31]. PPARG4 is limited to exon 1-6 common to all isotypes [33]. There have been numerous studies into the effects of genetic variability of PPARG gene sequence and expression, in both rodent models and human patients (Figure 2(b), Table 1).

Work initiated in rodent knockout models revealed that total PPARG$^{-/-}$ mutants display two independent lethal phases [23]. Firstly, PPARG deficiency interferes with terminal differentiation of the trophoblast cells and with placental vascularization, leading to myocardial thinning, and death by embryonic day 10. When PPARG null embryos are provided with a wild-type placenta, this cardiac defect was corrected permitting delivery, although postnatal pathologies (including multiple haemorrhages and lipodystrophy) resulted in lethality. To circumvent such restrictions, the Cre-loxP system can be applied, where Cre recombinase was under the control of the whey acidic protein (WAP) or mouse mammary tumour virus (MMTV) promoters. This causes PPARG gene deletions specific to secretory and hematopoietic tissues (alveolar epithelial cells of mammary tissue, salivary gland cells, oocytes, granulosa cells, megakaryocytes, and B- and T-cells) [50]. The results of this study revealed an important PPARG role in fertility: although the mutant mice appeared to ovulate normally, they exhibited reduced

Table 1: *Phenotypes and reproductive effects associated with PPARG mutations in mice and humans.* Abbreviations used: ART: artificial reproductive technology; BAT: brown adipose tissue; BMI: body mass index; HbA(1C): haemoglobin A1C; KO: knock-out; PCOS: polycystic ovary syndrome; T2DM: Type 2 Diabetes Mellitus; TG: Triglycerides; WAT: white adipose tissue.

Species	Genetic Abberation	Outcome	Effect on female fertility	Reference
Mouse	Global PPARG$^{-/-}$	Neonatal death	—	[23]
	Global PPARG$^{-/+}$	Improved insulin sensitivity	Fertile	[39]
	Mammary, epithelium, ovary, B- and T-cell null	Ovarian dysfunction and abrogated mammary development	30% of animals completely infertile, remainder had delayed conception, reduced litter size	[7]
	PPARG$^{hyp/hyp}$: WAT BAT, liver, and muscle null.	Normal birthweight but subsequent growth retardation, lipodystrophy, hyperlipideamia, and mild glucose intolerance	Heterozygote matings produce normal sized litters, but homozygote matings result in reduced litter size.	[40]
Human	Pro12Ala (34C > G), PPARG2 only.	Ala allele ↓ PPREs affinity, ↓ PPARG transactivation. ↑ Insulin sensitivity in some studies, conflicting reports on association with BMI.	Possible relationship with PCOS. In wider, non-PCOS population Ala allele associated with ↓ testosterone production	[41–43]
	Pro115Gln (344G > T), PPARG2 only.	Constitutively activated PPARG, ↑ adipocyte differentiation. Severe obesity, 3/4 subjects T2DM.	Fertility not assessed.	[44]
	His447His (1431C > T)	T allele may increase adipocyte differentiation. Presence of T allele associated with ↑ BMI, and insulin sensitivity.	T allele more common in PCOS compared to BMI-matched controls. T allele associated with ↓ testosterone.	[43, 45]
	Pro467Leu (1647C > T)	Mutation in LBD, ↓ coactivator recruitment and downstream transactivation. ↓ Basal gene activity. Lipodystrophy but normal BMI, severe insulin resistance and hypertension. One carrier (from 4) responsive to rosiglitazone therapy.	Oligomenorrhoea and hirtsutism, required ART for 1st pregnancy, complicated by pre-eclampsia and induced labour. 2nd pregnancy spontaneously conceived, with pre-eclampia, preterm emergency caesarean, and neonatal infant death.	[46, 47]
	Val290Met (1115G > A)	Mutation affects LBD, profound blockage of transcriptional activation. Similar phenotype to P467L. Unresponive to rosiglitazone therapy.	Primary amenorrhoea, hirsutism, acanthosis nigricans, and hypertension.	[46, 47]
	Phe388Leu (1164T > A)	↓ PPARG-ligand binding, ↓ basal transcriptional activity. Lipodystrophic and hypertensive with ↑ TG. Hyperinsulinemic, later T2DM.	Irregular menses, and bilateral polycystic ovaries treated with salpingo-oopherectomy. Prior to this carried two pregnancies.	[48]
	Arg397Cys (1273C > T)	Mutation in LBD, unknown effect on PPARG function. Lipodystrophic, ↑ TG and T2DM.	Hirsutism but no other indications of hyperandrogenism. Delayed menarche, but regular menses.	[49]

progesterone secretion as well as impaired implantation. Interestingly, fertility is affected even when the lesion in PPARG expression is restricted to extraovarian sites, as homozygote matings of $PPAR^{hyp/hyp}$ mutants, lacking PPARG expression in white and brown adipose tissue, liver, and muscle, had reduced litter size [40].

Examinations of naturally occurring human polymorphisms have focussed on susceptibility to Type II diabetes, insulin sensitivity, and obesity, and to date at least seven polymorphisms within the PPARG gene have been described.

The Pro12Ala polymorphism is located in exon2, and is only translated within the adipose tissue-, macrophage-, and colonic epithelium-specific PPARG2 isotype. The Pro12 allele is carried by approximately 85% of certain regional populations [51], and a single nucleotide mutation (C→G) leads to the substitution of an Ala amino acid [41]. PPARG protein produced by the Ala12 allele shows reduced in vitro affinity for PPAR response elements (PPREs) in target gene proximal promoters, and subsequently has reduced PPARG transactivation [41]. This PPARG SNP was extensively studied, following initial reports that it was strongly associated with bodyweight and insulin sensitivity [41], and the effect of the Ala12 mutation on PCOS symptoms has been closely studied, although some specific conclusions are difficult to reach. There are conflicting reports regarding the effect of this allele on BMI: either linked with increased BMI [45, 52–55], lower BMI [41, 56–59], or not associated at all [45, 60–64]. Current assumptions are that differential environmentalinteractions between populations can modify the function of this polymorphism. However, the relationship between Pro12Ala and insulin sensitivity appears more conclusive. Populations of women positive for Ala12 and PCOS have lower fasting insulin, reduced measures of systemic insulin resistance, lower insulin secretion, and lower hirsutism scores than women without the allele [54, 65, 66]. Consequently, the frequency of this allele is much lower in groups categorised as PCOS [54, 65, 67]. It appears that the Pro12Ala polymorphism of the PPARG gene may be a modifier of insulin resistance in women with PCOS, which can have a profound influence on fertility (see Section 4.1).

Another PPARG2-specific polymorphism is the rare Pro115Gln substitution in exon 3 that results in permanent, ligand independent activation [44]. This induces excessive adipocyte differentiation, and as a result the 4 individuals known to carry this (nonfamilial) SNP suffer extreme obesity [44], although present with only moderate metabolic complications including Type 2 Diabetes. The reproductive implications of hyperactive PPARG2 have not been addressed in these subjects.

All other reported polymorphisms are located in regions common to both PPARG1 and PPARG2. The His447His polymorphisms resulting from a C to T substitution at nucleotide 1431 in exon 6 is a silent polymorphism that encodes histidine with either allele [55]. Also referred to as the C161T polymorphism, it is proposed that this substitution may modulate expression of PPARG by altering mRNA processing or translation, leading to increased adipocyte differentiation. Subsequently, carriers of the T allele have elevated BMI. The T allele is also more common in women with PCOS compared to non-PCOS BMI-matched controls [45], and therefore has suspected involvement in the high incidence of obesity in PCOS population. However, both PCOS subjects and controls with T allele appear to be protected from other complicating symptoms of obesity, having better insulin sensitivity in addition to lower circulating testosterone.

The remaining polymorphisms are all extremely rare and restricted to single families.

Reported by Barroso et al. [46] and Savage et al. [47], there is a PPARG1 Pro467Leu substitution in the region required for ligand-dependent transactivation (PPARG2 residue 495) which results in impaired coactivator recruitment and downstream transactivation. This mutation also inhibits basal gene activity and has been found within 4 members of a single family spanning 3 generations. Medical histories reveal that in addition to lipodystrophy and hypertension (both frequently associated with PPARG mutations), the female carrier also experienced oligomenorrhea and hirsutism, and required ART intervention to conceive. This, and a subsequent spontaneously conceived pregnancy were both complicated with severe pre-eclampsia. Treatment of the male carrier with rosiglitazone (8 mg/day) was found to normalise chronic hyperglycaemia after 6 months, suggesting that the mutant PPARG protein is still able to be activated by exogenously sourced ligands, indicating the phenotypic profile of these subjects results from abnormal basal and endogenously activated PPARG activity.

Also identified by this study was a similarly positioned PPARG1 Val290Met mutation (PPARG2 residue 318) in a single female individual. This mutation results in a profound loss of PPARG function evidenced by both in vitro reporter gene activity, and in vivo response to rosiglitazone. Experiencing comparable metabolic complications to subjects with the Pro467Leu substitution, this individual also reported primary amenorrhoea, hirsutism, and acanthosis nigricans. Implications of these gynaecological and endocrine aberrations relating to conception and pregnancy have not been reported.

Another loss-of-function mutation is the phenylalanine to leucine substitution at position 388 (reported with respect to PPARG2, the substitution corresponds to residue 360 in PPARG1) found in 4 individuals from 3 generation of a single family [48]. Despite the reduction in normal PPARG function, concurrent treatment of one individual with both metformin and rosiglitazone (8 mg daily) provided effective glycemic control. Two of the affected individuals were female (46-year-old mother and her 22-year-old daughter), with the older individual experiencing a history of irregular menses and polycystic ovarian disease, eventually treated with bilateral salpingo-oophorectomy. At the time of study, the daughter did not have any significant medical problems (other than diet-controlled hyperinsulinemia and mild type IV hyperlipoproteinia), with regular menses and no polycystic ovarian pathology observed.

A heterozygous arginine to cysteine mutation at position 397 in PPARG1 (corresponding to residue 425 in PPARG2) was identified in a 64-year-old woman in 2002 by Argarwal and Garg [49]. Although the effect on PPARG functionality

has not been explicitly described, but the mutation lies in a region of the protein that forms a salt-bridge, and as a result, the mutated form may lack proper protein configuration. The Arg397Cys substitution was associated with lipodystrophy, elevated triglycerides, and early-onset Type 2 Diabetes. In addition, although pregnancy was never sought, moderate hirsutism as well as a history of delayed menarche (age 18) and subsequently irregular menstrual cycles were reported.

Overall, these studies demonstrate that PPARG precisely controls various aspect of systemic metabolism in humans. As female fertility is also disrupted in a significant number of these patients, it is likely that PPARG regulates female reproduction either directly, by intrinsic actions within reproductive organs such as the ovary, or indirectly via the myriad effects on metabolic tissues such as adipose and liver. To better define links between the metabolic and reproductive consequences observed in so many of these PPARG mutations, it would be interesting to recapitulate, in a tissue-specific manner, some of these PPARG genetic aberrations in mice.

3. LIGANDS

Together with expression of PPARG itself, availability of ligands is a primary regulating factor determining the ability of PPARG to influence target gene expression. Ligands can be produced endogenously, providing physiological significance, or sourced exogenously, as therapeutic factors given to target specific metabolic and reproductive symptoms.

3.1. Endogenous ligands: physiological function of PPARG

All PPARs bind and are activated by naturally occurring fatty acids and their metabolites [68], thus acting as fatty acid-activated receptors that function as key regulators of glucose and cholesterol metabolism. The precise nature of endogenous PPARG ligand binding and activation remains poorly defined and more research is needed in this area. However, the potential for important physiological ovarian PPARG activation is considerable, as many natural ligands have been shown to be present within the ovary, and produced locally by ovarian cells. Included in this list are ω3- and ω6-polyunsaturated fatty acids (PUFAs) such as the essential fatty acids linoleic acid, linolenic acid, arachidonic acid, and eicosapentanoic acid ([69] and reviewed [34]). Additional PPARG agonists such as prostaglandin metabolites of these substances and immunologically-derived eicosanoids are also produced within the ovarian environment in a hormonally regulated manner, with elevated production as ovulation progresses [70–73]. It is possible that PPARG may have a role in the feed-forward production of eicosanoid ligands, based on identification of a PPRE in the prostaglandin-endoperoxide synthase 2 (a.k.a. COX-2) promoter [74], which would facilitate amplified production of pro-ovulatory prostaglandins.

3.2. Exogenous ligands: therapeutic application of PPARG activation

As information emerges regarding the endogenous roles for naturally activated PPARG within the ovarian follicular environment, other evidence of PPARG involvement with ovarian function comes from reports utilising synthetic PPARG ligands, specifically, administration of TZDs to women diagnosed with PCOS (Table 2).

PCOS is the leading cause of infertility and menstrual irregularities in women of reproductive age and is characterised by chronic hyperandrogenic anovulation [90]. This is thought to be due, in general, to hypothalamic-pituitary axis dysregulation causing elevated basal LH levels that overstimulate cells of the theca interna [91]. Insulin resistance also appears to contribute to the syndrome in many instances [92], as the pituitary responds to elevated plasma levels of insulin to augment LH release [91].

The potential merits of applying TZDs to improve reproductive outcomes in infertile PCOS women was first demonstrated by Azziz et al. [93]. Since then, treatment of PCOS patients with the TZDs rosiglitazone or pioglitazone have been shown to not only improve insulin action in peripheral tissues, attenuate hyperinsulinemia, and lower circulating levels of lipids [92, 94], but also to improve a range of reproductive outcomes particularly circulating sex hormone levels, and ovulation rate [77, 88, 90, 95–97] (see recent reports summarised in Table 2).

The beneficial effects of TZDs on ovarian PCOS symptoms were first attributed to improvements in defective insulin action and secretion [92]. However, actions upon various ovarian cells directly illustrated both in vitro [1, 9, 14, 98, 99] and in vivo [100, 101] confirms a direct interaction between these compounds and ovarian PPARG.

Particular focus has been directed upon the effect of PPARG activation on the synthesis of ovarian steroid hormones and the expression of many rate-limiting steroidogenic enzymes has been investigated.

(1) Steroidogenic acute regulatory protein (StAR): facilitates that rapid mobilization of cholesterol for initial catalysis to pregnenolone by the P450-side chain cleavage enzyme located within the mitochondria [102]. It has been recently reported that both rosiglitazone and pioglitazone significantly up regulate StAR protein synthesis by human granulosa cells in vitro [103].

(2) 3β-hydroxysteroid dehydrogenase (3β-HSD): catalyses the conversion of pregnenolone to progesterone by luteal cells [104]. Work on porcine granulosa cells has found that troglitazone competitively inhibits 3β-HSD enzyme activity within these cells [99].

(3) Steroid 17-alpha-hydroxylase (P450c17): converts progesterone to androgen within ovarian theca cells [105]. Conflicting reports have arisen regarding the effect of TZDs on the expression and activity of this enzyme, many of which may be artefacts of various culture conditions. P450c17 mRNA production has been found to increase following porcine thecal cell exposure to TZDs [8], whilst other reports indicate suppression

Table 2: *Summary of reports published within the past 2 years on the use of PPARG activating agents for reproductive symptoms.* Abbreviations used: AUC, area under the curve; BMI body mass index; CC clomiphene citrate; DHEA-S dehydroepiandrosterone sulfate; E2, estradiol; FAI, free androgen index; FSH, follicle-stimulating hormone; GnRH, gonadotropin releasing hormone; HbA(1C), haemoglobin A1C; HDL-C, high density lipoprotein-cholesterol; HOMA, homeostasis model of assessment for insulin sensitivity; IGF1 insulin-like growth factor 1; IGFBP-1/3, insulin-like growth factor binding protein 1 or 3; LDL-C, low density lipoprotein-cholesterol; LH, luteinizing hormone; OGTT, oral glucose tolerance test; PCOS, polycystic ovary syndrome; QUICKI, quantitative insulin-sensitivity check index; SHBG, sex hormone binding globulin; T, testosterone; WHR, waist to hip ratio.

Reference:	Rautio et al. [75] and Rautio et al. [76]
Patient profile:	Overweight but not obese PCOS ($n = 30$)
PPAR agonist:	Rosiglitazone (4 mg once daily for 2 weeks then 4 mg twice daily for 16 weeks)
Metabolic outcomes:	Serum C-reactive protein levels, leukocyte count, and alanine aminotransferase enzyme activity decreased, but lipid and blood pressure did not change. Glucose tolerance and peripheral insulin response normalized in the rosiglitazone group.
Reproductive outcomes:	Rosiglitazone improved menstrual cyclicity, SHBG levels; and decreased serum levels of androstenedione, 17-hydroxyprogesterone (17-OHP), DHEA and DHEA-S.
Reference:	Rouzi and Ardawi [77]
Patient profile:	Obese PCOS ($n = 12$)
PPAR agonist:	Rosiglitazone (4 mg twice daily for 3 cycles, CC administered for 5 days starting 3 days after rosiglitazone initiated)
Metabolic outcomes:	No changes in fasting plasma glucose or HbA(1C) or IGFBP-3 values. Fasting serum insulin, DHEA-S, androstenedione, and IGF-1 levels decreased significantly and IGFBP-1 exhibited significant increases.
Reproductive outcomes:	Total-T, free-T, LH, and SHBG decreased. Follicular development and ovulation rate increased, trend for increased pregnancy rate in group receiving short-term administration of rosiglitazone compared to matched control receiving metformin.
Reference:	Mitkov et al. [78]
Patient profile:	Obese, insulin resistant PCOS ($n = 15$)
PPAR agonist:	Rosiglitazone (4 mg/day for 12 weeks)
Metabolic outcomes:	Hyperinsulinemia and insulin resistance normalized.
Reproductive outcomes:	Total-T and FAI profile tended to normalise. Number of women with oligomenorrhea was reduced by 67%
Reference:	Cataldo et al. [79]
Patient profile:	Insulin resistant PCOS ($n = 11$–16/group)
PPAR agonist:	Rosiglitazone (2, 4 or 8 mg/day for 12 weeks)
Metabolic outcomes:	Steady state plasma glucose declined and hyperinsulinemia fell in a dose-dependent manner.
Reproductive outcomes:	Serum LH, total-T, and free-T were unchanged; SHBG increased. Ovulation occurred in 55%, without significant dose dependence. Before and during treatment, ovulators on rosiglitazone had lower circulating insulin and free-T and higher SHBG than nonovulators.
Reference:	Lemay et al. [80]
Patient profile:	Overweight, insulin resistant PCOS ($n = 15$)
PPAR agonist:	Rosiglitazone (4 mg/day for 6 months)
Metabolic outcomes:	Plasma insulin, insulin resistance indices and insulin AUC in response to OGTT all decreased compared to controls receiving antiandrogenic estrogen-progestin. Effect on lipids was limited.
Reproductive outcomes:	No significant effect on androgens or hirsutism.
Reference:	Garmes et al. [81]
Patient profile:	Obese insulin resistant PCOS ($n = 15$)
PPAR agonist:	Pioglitazone (30 mg/day for 8 weeks)
Metabolic outcomes:	Insulin response to OGTT significantly decreased.
Reproductive outcomes:	Total-T and free-T levels decreased, SHBG increased, and LH response to GnRH stimulation decreased.
Reference:	Yilmaz et al. [82–84]
Patient profile:	Obese or lean PCOS ($n = 20$ obese, $n = 20$ lean)
PPAR agonist:	Rosiglitazone (4 mg/day for 12 weeks)
Metabolic outcomes:	Indices of oxidative stress improved. HOMA, insulin AUC, fasting insulin and C-peptide levels decreased significantly. Glucose/insulin ratio and BMI increased

Table 2: Continued.

Reference:	Rautio et al. [75] and Rautio et al. [76]
Patient profile:	Overweight but not obese PCOS ($n = 30$)
PPAR agonist:	Rosiglitazone (4 mg once daily for 2 weeks then 4 mg twice daily for 16 weeks)
Metabolic outcomes:	Serum C-reactive protein levels, leukocyte count, and alanine aminotransferase enzyme activity decreased, but lipid and blood pressure did not change. Glucose tolerance and peripheral insulin response normalized in the rosiglitazone group.
Reproductive outcomes:	Rosiglitazone improved menstrual cyclicity, SHBG levels; and decreased serum levels of androstenedione, 17-hydroxyprogesterone (17-OHP), DHEA and DHEA-S.
Reproductive outcomes:	Seurm levels of free-T, androstenedione, and DHEA-S decreased significantly. Menstrual disturbances improved in 61.5% of lean and 53.8% of obese patients. In a second cohort of patients, menstrual cycles became regular in 87.8%.
Reference:	Tarkun et al. [85]
Patient profile:	Young, lean PCOS ($n = 31$)
PPAR agonist:	Rosiglitazone (4 mg/day for 12 months)
Metabolic outcomes:	Fasting insulin and insulin resistance indices significantly improved. No changes in BMI, waist circumference, serum total cholesterol, or LDL-C. Serum C-reactive protein levels decreased; and endothelium-dependent vascular responses improved.
Reproductive outcomes:	Significant decreases in serum T, although no change in FSH and LH levels. Hirsutism score decreased significantly after treatment. 77.4% of women reverted to regular menstrual cycles. Levels of SHBG increased significantly after treatment.
Reference:	Dereli et al. [86]
Patient profile:	Nonobese PCOS ($n = 20$/group)
PPAR agonist:	Rosiglitazone (2 mg/day or 4 mg/day for 8 months)
Metabolic outcomes:	75% of women in the 2 mg group and 95% in the 4 mg group achieved normal glucose tolerance. Improved insulin resistance in a dose-related fashion, without adverse events or liver enzyme elevations.
Reproductive outcomes:	Decreased free-T levels were better in the 4 mg group than the 2 mg group, and 70% of women in the 2 mg group and 85% of women in the 4 mg group achieved ovulatory menses.
Reference:	Mehta et al. [87]
Patient profile:	Obese PCOS ($n = 9$)
PPAR agonist:	Pioglitazone (45 mg/day for 20 weeks)
Metabolic outcomes:	Significant improvement in insulin sensitivity
Reproductive outcomes:	LH levels, LH pulse frequency and amplitude, as well as gonadotropin responses to GnRH were not influenced.
Reference:	Ortega-González et al. [88]
Patient profile:	Obese, insulin resistant PCOS ($n = 25$)
PPAR agonist:	Pioglitazone (30 mg/day for 6 months)
Metabolic outcomes:	Body weight, BMI, and WHR increased significantly. Fasting insulin and insulin AUC during a 2-h OGTT decreased. Insulin resistance decreased and insulin sensitivity increased after treatment with either pioglitazone or metformin received by control group.
Reproductive outcomes:	Hirsutism, free-T and androstenedione declined to a similar extent after treatment with either drug. Treatment with both drugs was associated with the occurrence of pregnancy
Reference:	Sepilian and Nagamani [89]
Patient profile:	Obese insulin resistant PCOS ($n = 12$)
PPAR agonist:	Rosiglitazone (4 mg/day for 6 months)
Metabolic outcomes:	Fasting insulin, insulin AUC, fasting glucose, and glucose AUC significantly decreased. No significant change in BMI
Reproductive outcomes:	Both total-T, free-T and DHEA-S levels decreased significantly. No significant change in LH levels. Levels of SHBG increased significantly after treatment, 91.7% of women reverted to regular ovulatory cycles during the treatment period

of enzymatic expression and/or activity in primary porcine thecal cells or human cell lines [106–108].

(4) P450 aromatase (P450arom): aromatises androgen precursor to estradiol, and is expressed by ovarian granulosa [109] and luteal [110] cells. Although there is no correlation between the expression of the P450arom enzyme and PPARG itself during folliculogenesis, many reports have described the down-regulation of P450arom following TZD exposure in human ovarian cell cultures [13, 111–113].

Taken together, these findings provide strong evidence for the direct effect of TZD administration and PPARG activation on ovarian hormonal synthesis and secretion. Specifically the following.

(1) Androgen: TZDs found to inhibit LH- and insulin-stimulated androgen biosynthesis by purified porcine thecal [108], and mixed human ovarian [98] cells. They have also been found to reduce plasma testosterone levels in women with PCOS [76, 77, 81–84, 86, 89, 91, 97, 114, 115].

(2) Estrogen: While it is accepted that TZDs indeed influence estrogen secretion, estrogenic responses to TZDs appear to be dependent on confounding factors such as species, age, and endocrine setting. For instance, TZDs have been found to increase estradiol secretion [2], and decrease estradiol production [116]. PPARG activation by TZDs and phthalate toxins are believed to mediate the antiestrogenic effects of these agents in cultured rat granulosa cells [116], and TZDs have also been found to suppress stimulated estradiol secretion in human granulosa cell cultures [98].

(3) Progesterone: As for estrogen, progesterone responses to PPARG activation via natural or endogenous ligands are unclear, and are probably regulated by species, and stage of folliculogenesis. Most publications investigating a range of species, including primary bovine, ovine, porcine, or rodent cell cultures, report increases in progesterone secretion following administration of PPARG activators in vitro [2, 8–10], whilst some others suggest inhibition of stimulated progesterone secretion by porcine granulosa cells [14].

The net influence of TZD treatment on ovarian PPARG activation and subsequent steroidogenesis in vivo remains poorly defined across all species investigated. The most conclusive evidence for an advantageous outcome on hormonal (specifically androgen) profile following treatment is observed in women with PCOS, as overviewed in Table 2. As a result, increasing attention may be paid towards the application of these drugs in such conditions of significant hormonal perturbations.

Rosiglitazone and pioglitazone are currently listed as a Pregnancy Category C drug (i.e., not tested for use during human pregnancy), and some side effects of TZD administration, such as weight gain, fluid retention (reviewed in [117]), and possible bone demineralisation [118], preclude their widespread use during pregnancy. However, in vitro treatment of 2 cell mouse embryos, or in vivo treatment of pregnant mice with rosiglitazone was not found to impact upon normal blastocyst development, or litter rates and sizes [119]. In situations where conception has occurred following TZD treatment for PCOS, no adverse fetal outcomes have been observed [88, 96, 120]. Also in a recent study, examining tissue obtained from women with scheduled pregnancy terminations, it was found that placental transfer of maternally administered rosiglitazone to fetal tissues is minimal in the first 10 weeks of pregnancy [121].

4. MECHANISMS: PPARG REGULATION OF METABOLIC AND IMMUNE FACTORS INFLUENCING FEMALE FERTILITY

PPARG is known to regulate many pathways involving insulin sensitivity, glucose metabolism, adipokine signalling, lipid uptake and metabolism, and secretion of inflammatory mediators. As a result, PPARG is being revealed as a key mediator of the fundamental metabolic and immune contributions that are required for normal female fertility.

4.1. Insulin sensitivity

Normal insulin sensitivity and subsequently efficient metabolism of glucose are essential for healthy reproduction in the female. Conditions of hyperinsulinemia can interfere with normal ovarian cell function or be indirectly associated with other hormonal conditions detrimental to optimal fertility [122–124]. Also, exposure to high levels of glucose can have a deleterious effect on the oocyte [125, 126]. By normalising peripheral insulin signalling, PPARG activation can circumvent many of these adverse effects of hyperinsulinemia, as well as those detrimental outcome associated with persistently elevated blood glucose levels.

The genetic studies detailed above, and the pharmacokinetics of TZD treatment improving insulin sensitivity are both consistent with a direct role for PPARG in the regulation of cellular insulin utilization. Despite this, it remains to be determined exactly how TZD treatment and subsequent PPARG activation impacts gene expression directly related to insulin signalling and glucose uptake (through genes such as the insulin receptor (IR), IR-substrates, and glucose transporters), as a range of conflicting results have emerged. Suggested mechanisms include increases in glucosetransport protein 4 (GLUT4), stimulation of phosphatidyl-3-kinase andmodified phosphorylation of insulin receptor substrates [127–133]. In addition, it is well accepted that activation of PPARG does improve not only basal hepatic glucose secretion, but also peripheral insulin-stimulated glucose uptake, potentially indirectly via reduction of FFA, TNFα, plasminogen activator inhibitor-1, and other autocrine/endocrine signalling molecules which otherwise interfere with efficient insulin signalling (reviewed in [134]). In this way, PPARG activation may improve female infertility exacerbated by obesity and insulin resistance [25, 135–141].

New reports are also describing some of the first investigations into the ovarian-specific responses to TZD that facilitate insulin sensitivity in this tissue. The work of Seto-Young et al. [103] has shown that ovarian cells directly respond to

TZDs to increase transcription of insulin signalling components including IR alpha and beta subunits and IRS-1, which would subsequently provide more efficient signal transduction and cellular response to insulin.

4.2. Adipokines: leptin and adiponectin

Produced primarily by adipose tissue, leptin and adiponectin are "adipokines" with contrasting actions on insulin sensitivity. Whilst other adipokines such as visfatin and retinol-binding protein 4 (RBP-4) are also linked with insulin sensitivity [142, 143] and the incidence of PCOS [144, 145], leptin and adiponectin are of particular interest to those investigating female reproduction as it is known their presence can be detected by ovarian cells which express leptin and adiponectin receptors. In addition, although only the adiponectin promoter has been shown to contain a PPRE [146], transcriptional activity of both leptin and adiponectin genes is known to be decreased and increased, respectively, in the presence of PPARG-activating ligands [147–151]. In this way, they can operate as secondary messengers of signals initiated by PPARG activation.

Leptin receptors are present in the granulosa and thecal layers of the ovary [152, 153], and have been shown to be cyclically regulated [154]. Leptin appears to influence ovarian gonadotropin and steroid secretion [152, 153, 155], and affect oocyte quality and developmental potential [156, 157].

Adiponectin receptors AdipoR1 and AdipoR2 are also both expressed by ovarian tissue [158] and adiponectin itself has been identified within the follicular fluid of developed follicles in similar concentrations to that observed in the serum [159]. Adiponectin appears to be involved in many processes including those essential for ovulation, such as induction of COX-2 and prostaglandin E synthase expression in ovarian granulosa cells [159].

As the entire range of leptin and adiponectin effects on ovarian cellular functions, including the outcomes of PPARG activation (including enhancement of insulin sensitivity), are gradually established, it is likely we will find that the improvements to reproductive profiles and ovarian function of sub-fertile or infertile women treated with TZDs are mediated, at least in part, through modulation of these two adipokines.

4.3. Lipid uptake: CD36 and SCARB1

PPARG has a critical role in the regulation of adipocyte differentiation [94]. Among the best characterised PPARG target genes are those involved in lipid metabolism, including phosphoenolpyruvate carboxykinase [160], lipoprotein lipase [161], fatty acid binding protein [162, 163], and CD36 and SCARB1 [164, 165]. CD36 and SCARB1, class B scavenger receptors that mediate the endocytosis or selective cholesterol uptake from oxLDL and HDL lipoproteins, are also both strongly expressed by the ovary. The CD36 antigenis highly expressed by granulosa cells of preantral and earlyantral follicles, with moderatestaining also evident in the vascular thecal layers [166].In this context, CD36 has been reported as a facilitator of thrombospondins-1 and -2 activities [166, 167], influencing cell adhesion, wound healing, and angiogenesis [168, 169]; important components of the tissue and cellular changes that occur during the ovarian cycle. CD36 is upregulated following activation of PPARG in macrophages [164, 170], and a summary of PPARG control of gene expression [171] suggested this might act as a positive feedback mechanism, such that more potential PPARG ligands can be imported, enhancing expression of both PPARG and CD36.

Ovarian SCARB1 expression appears to be strongly associated with HDL-cholesterol ester requirement for production of androgen for aromatase-mediated conversion to estradiol by the granulosa cells, and progesterone synthesis by luteal cells. Thecal cells consistently express high levels of SCARB1 at all stages of both healthy and atretic follicle development [172], and high expression is also found within luteal structures [173].

In these respects, PPARG activation may have profound influence on ovarian function through the regulation of these genes or others regulating lipid metabolism, by affecting availability of substrate for hormone synthesis, and the remodelling of tissue structures required for oocyte release, luteinization, and luteolysis.

4.4. Suppression of chronic inflammation

An important role for PPARG is the suppression of immune cell synthesis and secretion of proinflammatory mediators [174–182] (reviewed [183–185]). The role of the immune system in female fertility is critical, both systemically, and locally at the ovarian level.

In addition, there are also interesting correlations between the development of adiposity, insulin resistance and, chronic inflammation. Increased serum concentrations of TNF, NO, and IL-6 are strongly associated with obesity [186, 187], and proinflammatory cytokines sourced from adipose tissue including TNF, and IL-6 are among several important factors that participate in the development of insulin resistance and type 2 diabetes mellitus [188–191]. Interestingly, together with central adiposity and insulin resistance, we also find aspects of systemic inflammation independently associated with impaired female fertility and PCOS [192, 193]. PPARG is implicated in improvements to the systemic inflammation observed in obese and insulin resistant individuals treated with TZDs. These studies describe reductions in serum C-reactive protein, IL-6, and soluble TNF receptor 2 [194–198]. Other studies investigating the chronically inflamed profile of PCOS patients support these findings, reporting that in addition to restoring menstrual cyclicity and improving markers of hyperandrogensism, TZD treatment is able to lower circulating C-reactive protein levels and the number of circulating leukocytes [75, 85].

4.5. Ovarian macrophages

Macrophages, dendritic cells, lymphocytes, and neutrophils have unique roles in the context of ovarian physiology, and are essential for the normal regulation of ovulation and control of the reproductive cycle [5, 199–201]. Macrophage

distribution and numbers within the ovary varies across the cycle, influenced by gonadotrophins and ovarian steroidogenic hormones. Resident macrophages are present in the theca and stroma of the ovary during the late stages of folliculogenesis [202]. Once the LH surge commences prior to ovulation, there is a massive recruitment of new leukocytes from the circulation into the theca of the preovulatory follicle [202, 203], where they function to release proinflammatory cytokines and mediators assisting the breakdown of the ovarian epithelium at ovulation. Their presence persists until after ovulation, further increasing in number in the developing and regressing corpus luteum [204].

Ovarian macrophages maintain high levels of PPARG transcript expression until a significant reduction in response to the proovulatory LH surge [1]. Immediately following ovulation, expression is restored to high preovulatory levels [1]. In vitro treatment of purified ovarian macrophages with the TZD troglitazone has been shown to significantly alter proinflammatory gene expression [1]. Specifically, these cells respond to TZD exposure by significantly suppressing mRNA production of NOS2 (or inducible Nitric Oxide Synthase, iNOS), the enzyme that catalyses the reaction producing the potent vasodilator, nitric oxide (NO). In the human, NO seems to direct follicular selection and maturation [205], and application of this NO property to IVF patients, deemed "poor responders", has been found to increase the number of oocytes retrieved [206]. This is an indication that recruited and specialized ovarian macrophages can potentially respond directly to TZDs administered systemically, and can regulate the availability of ovulatory mediators. Such responses parallel the anti-inflammatory effects of PPARG activation in nonovarian-activated macrophages [171], but was here found to be specific to macrophages closely associated with the ovarian environment (distinct to those located in the peritoneal cavity for instance). This illustrates the unique influence of the ovarian milieu on normal PPARG function and effects.

5. CONCLUSIONS

Many diverse endocrine and metabolic components profoundly influence female fertility, including hormone production as well as the development and ovulation of healthy oocytes. The role of PPARG in these events is two-fold. PPARG activation of transcription has outcomes operating both directly within the ovarian structure itself, and also indirectly through influences on other tissue systems such as the adipose tissue and immune cells (Figure 3). In this way, PPARG controls key signals regulating the capacity for normal reproduction. As PPARG is able, and required, to regulate many of these actions, it is important that the roles of PPARG be carefully considered as new concepts develop regarding the effects of dietary supplements such as PUFAs, which are PPARG ligands, and the consequences of increased immunological activation, such as occurs during obesity. As the health crisis surrounding the obesity epidemic widens to include the damaging effects on female fertility, it is important to remember the systemic implications of metabolism and immune regulation on female fertility, and to consider the role of PPARG in coordinating these contributions. Tremendous opportunity exists for those interested in elucidating further the exciting interactions between PPARG and female fertility. Publication of the most extensive list to date of all genes containing potential PPREs in their promoter regions [207] will provide a valuable tool for such research, as many identified genes have known functions within the context of ovarian physiology and pathology, in addition to characterized roles in other tissues, including macrophages and adipose tissue.

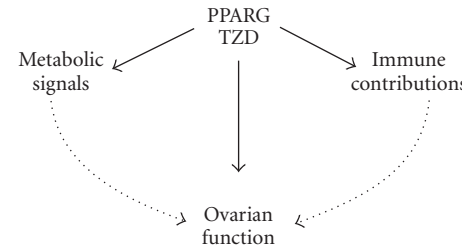


Figure 3: Schematic summarising the developing concept of PPARG influence on ovarian function and female fertility. PPARG is able to strongly influence the activity of ovarian cells directly, in particular steroidogenesis and tissue remodelling. In addition, PPARG can further influence ovarian function via regulation of external metabolic signals and immune cell contributions.

REFERENCES

[1] C. E. Minge, N. K. Ryan, K. H. Van Der Hoek, R. L. Robker, and R. J. Norman, "Troglitazone regulates peroxisome proliferator-activated receptors and inducible nitric oxide synthase in murine ovarian macrophages," *Biology of Reproduction*, vol. 74, no. 1, pp. 153–160, 2006.

[2] C. M. Komar, O. Braissant, W. Wahli, and T. E. Curry Jr., "Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period," *Endocrinology*, vol. 142, no. 11, pp. 4831–4838, 2001.

[3] C. M. Komar, "Peroxisome proliferator-activated receptors (PPARs) and ovarian function—implications for regulating steroidogenesis, differentiation, and tissue remodeling," *Reproductive Biology and Endocrinology*, vol. 3, no. 41, 2005.

[4] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[5] R. Wu, K. H. Van der Hoek, N. K. Ryan, R. J. Norman, and R. L. Robker, "Macrophage contributions to ovarian function," *Human Reproduction Update*, vol. 10, no. 2, pp. 119–133, 2004.

[6] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[7] Y. Cui, K. Miyoshi, E. Claudio, et al., "Loss of the peroxisome proliferation-activated receptor γ (PPARγ) does not affect mammary development and propensity for tumor formation but leads to reduced fertility," *Journal of Biological Chemistry*, vol. 277, no. 20, pp. 17830–17835, 2002.

[8] P. D. Schoppee, J. C. Garmey, and J. D. Veldhuis, "Putative activation of the peroxisome proliferator-activated receptor γ impairs androgen and enhances progesterone biosynthesis in primary cultures of porcine theca cells," *Biology of Reproduction*, vol. 66, no. 1, pp. 190–198, 2002.

[9] P. Froment, S. Fabre, J. Dupont, et al., "Expression and functional role of peroxisome proliferator-activated receptor-γ in ovarian folliculogenesis in the sheep," *Biology of Reproduction*, vol. 69, no. 5, pp. 1665–1674, 2003.

[10] B. Löhrke, T. Viergutz, S. K. Shahi, et al., "Detection and functional characterisation of the transcription factor peroxisome proliferator-activated receptor γ in lutein cells," *Journal of Endocrinology*, vol. 159, no. 3, pp. 429–439, 1998.

[11] H. Sundvold, A. Brzozowska, and S. Lien, "Characterisation of bovine peroxisome proliferator-activated receptors γ1 and γ2: genetic mapping and differential expression of the two isoforms," *Biochemical and Biophysical Research Communications*, vol. 239, no. 3, pp. 857–861, 1997.

[12] K. G. Lambe and J. D. Tugwood, "A human peroxisome-proliferator-activated receptor-γ is activated by inducers of adipogenesis, including thiazalidinedione drugs," *European Journal of Biochemistry*, vol. 239, no. 1, pp. 1–7, 1996.

[13] Y.-M. Mu, T. Yanase, Y. Nishi, et al., "Insulin sensitizer, troglitazone, directly inhibits aromatase activity in human ovarian granulosa cells," *Biochemical and Biophysical Research Communications*, vol. 271, no. 3, pp. 710–713, 2000.

[14] S. Gasic, Y. Bodenburg, M. Nagamani, A. Green, and R. J. Urban, "Troglitazone inhibits progesterone production in porcine granulosa cells," *Endocrinology*, vol. 139, no. 12, pp. 4962–4966, 1998.

[15] C. M. Komar and T. E. Curry Jr., "Localization and expression of messenger RNAs for the peroxisome proliferator-activated receptors in ovarian tissue from naturally cycling and pseudopregnant rats," *Biology of Reproduction*, vol. 66, no. 5, pp. 1531–1539, 2002.

[16] M. Mohan, J. R. Malayer, R. D. Geisert, and G. L. Morgan, "Expression patterns of retinoid X receptors, retinaldehyde dehydrogenase, and peroxisome proliferator activated receptor γ in bovine preattachment embryos," *Biology of Reproduction*, vol. 66, no. 3, pp. 692–700, 2002.

[17] C. Dreyer and H. Ellinger-Ziegelbauer, "Retinoic acid receptors and nuclear orphan receptors in the development of Xenopus laevis," *International Journal of Developmental Biology*, vol. 40, no. 1, pp. 255–262, 1996.

[18] C. M. Komar and T. E. Curry Jr., "Inverse relationship between the expression of messenger ribonucleic acid for peroxisome proliferator-activated receptor γ and P450 side chain cleavage in the rat ovary," *Biology of Reproduction*, vol. 69, no. 2, pp. 549–555, 2003.

[19] A. Chawla, E. J. Schwarz, D. D. Dimaculangan, and M. A. Lazar, "Peroxisome proliferator-activated receptor (PPAR) γ: adipose-predominant expression and induction early in adipocyte differentiation," *Endocrinology*, vol. 135, no. 2, pp. 798–800, 1994.

[20] A. Chawla, Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans, "PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation," *Nature Medicine*, vol. 7, no. 1, pp. 48–52, 2001.

[21] P. Tontonoz, E. Hu, and B. M. Spiegelman, "Stimulation of adipogenesis in fibroblasts by PPARγ2, a lipid-activated transcription factor," *Cell*, vol. 79, no. 7, pp. 1147–1156, 1994.

[22] E. D. Rosen, P. Sarraf, A. E. Troy, et al., "PPARγ is required for the differentiation of adipose tissue in vivo and in vitro," *Molecular Cell*, vol. 4, no. 4, pp. 611–617, 1999.

[23] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[24] M. J. Davies, "Evidence for effects of weight on reproduction in women," *Reproductive BioMedicine Online*, vol. 12, no. 5, pp. 552–561, 2006.

[25] R. J. Norman and A. M. Clark, "Obesity and reproductive disorders: a review," *Reproduction, Fertility and Development*, vol. 10, no. 1, pp. 55–63, 1998.

[26] Y.-M. Mu, T. Yanase, Y. Nishi, et al., "Saturated FFAs, palmitic acid and stearic acid, induce apoptosis in human granulosa cells," *Endocrinology*, vol. 142, no. 8, pp. 3590–3597, 2001.

[27] R. Jorritsma, M. L. César, J. T. Hermans, C. L. J. J. Kruitwagen, P. L. A. M. Vos, and T. A. M. Kruip, "Effects of non-esterified fatty acids on bovine granulosa cells and developmental potential of oocytes in vitro," *Animal Reproduction Science*, vol. 81, no. 3-4, pp. 225–235, 2004.

[28] T. Vanholder, J. L. M. R. Leroy, A. Van Soom, et al., "Effect of non-esterified fatty acids on bovine granulosa cell steroidogenesis and proliferation in vitro," *Animal Reproduction Science*, vol. 87, no. 1-2, pp. 33–44, 2005.

[29] M. Mitchell, D. T. Armstrong, R. L. Robker, and R. J. Norman, "Adipokines: implications for female fertility and obesity," *Reproduction*, vol. 130, no. 5, pp. 583–597, 2005.

[30] A. M. Bodles, V. Varma, A. Yao-Borengasser, et al., "Pioglitazone induces apoptosis of macrophages in human adipose tissue," *Journal of Lipid Research*, vol. 47, no. 9, pp. 2080–2088, 2006.

[31] L. Fajas, J.-C. Fruchart, and J. Auwerx, "PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter," *FEBS Letters*, vol. 438, no. 1-2, pp. 55–60, 1998.

[32] P. Tontonoz, R. A. Graves, A. I. Budavari, et al., "Adipocyte-specific transcription factor ARF6 is a heterodimeric complex of two nuclear hormone receptors, PPAR γ and RXR α," *Nucleic Acids Research*, vol. 22, no. 25, pp. 5628–5634, 1994.

[33] H. Sundvold and S. Lien, "Identification of a novel peroxisome proliferator-activated receptor (PPAR) γ promoter in man and transactivation by the nuclear receptor RORα1," *Biochemical and Biophysical Research Communications*, vol. 287, no. 2, pp. 383–390, 2001.

[34] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[35] R. Mukherjee, L. Jow, G. E. Croston, and J. R. Paterniti Jr., "Identification, characterization, and tissue distribution of human peroxisome proliferator-activated receptor (PPAR) isoforms PPARγ2 versus PPARγ1 and activation with retinoid X receptor agonists and antagonists," *Journal of Biological Chemistry*, vol. 272, no. 12, pp. 8071–8076, 1997.

[36] L. Fajas, D. Auboeuf, E. Raspé, et al., "The organization, promoter analysis, and expression of the human PPARγ gene," *Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[37] Y.-S. Tsai and N. Maeda, "PPARγ: a critical determinant of body fat distribution in humans and mice," *Trends in Cardiovascular Medicine*, vol. 15, no. 3, pp. 81–85, 2005.

[38] M. Stumvoll and H. Häring, "The peroxisome proliferator-activated receptor-γ2 Pro12Ala polymorphism," *Diabetes*, vol. 51, no. 8, pp. 2341–2347, 2002.

[39] P. D. G. Miles, Y. Barak, W. He, R. M. Evans, and J. M. Olefsky, "Improved insulin-sensitivity in mice heterozygous for PPAR-γ deficiency," *Journal of Clinical Investigation*, vol. 105, no. 3, pp. 287–292, 2000.

[40] H. Koutnikova, T.-A. Cock, M. Watanabe, et al., "Compensation by the muscle limits the metabolic consequences of lipodystrophy in PPARγ hypomorphic mice," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 24, pp. 14457–14462, 2003.

[41] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[42] J. Masugi, Y. Tamori, H. Mori, T. Koike, and M. Kasuga, "Inhibitory effect of a proline-to-alanine substitution at codon 12 of peroxisome proliferator-activated receptor-γ 2 on thiazolidinedione-induced adipogenesis," *Biochemical and Biophysical Research Communications*, vol. 268, no. 1, pp. 178–182, 2000.

[43] H. J. Antoine, M. Pall, B. C. Trader, Y.-D. I. Chen, R. Azziz, and M. O. Goodarzi, "Genetic variants in peroxisome proliferator-activated receptor γ influence insulin resistance and testosterone levels in normal women, but not those with polycystic ovary syndrome," *Fertility and Sterility*, vol. 87, no. 4, pp. 862–869, 2007.

[44] M. Ristow, D. Müller-Wieland, A. Pfeiffer, W. Krone, and C. R. Kahn, "Obesity associated with a mutation in a genetic regulator of adipocyte differentiation," *New England Journal of Medicine*, vol. 339, no. 14, pp. 953–959, 1998.

[45] F. Orio Jr., G. Matarese, S. Di Biase, et al., "Exon 6 and 2 peroxisome proliferator-activated receptor-γ polymorphisms in polycystic ovary syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 12, pp. 5887–5892, 2003.

[46] I. Barroso, M. Gurnell, V. E. F. Crowley, et al., "Dominant negative mutations in human PPARγ associated with severe insulin resistance, diabetes mellitus and hypertension," *Nature*, vol. 402, no. 6764, pp. 880–883, 1999.

[47] D. B. Savage, G. D. Tan, C. L. Acerini, et al., "Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-γ," *Diabetes*, vol. 52, no. 4, pp. 910–917, 2003.

[48] R. A. Hegele, H. Cao, C. Frankowski, S. T. Mathews, and T. Leff, "*PPARG* F388L, a transactivation-deficient mutant, in familial partial lipodystrophy," *Diabetes*, vol. 51, no. 12, pp. 3586–3590, 2002.

[49] A. K. Agarwal and A. Garg, "A novel heterozygous mutation in peroxisome proliferator-activated receptor-γ gene in a patient with familial partial lipodystrophy," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 1, pp. 408–411, 2002.

[50] T. E. Akiyama, S. Sakai, G. Lambert, et al., "Conditional disruption of the peroxisome proliferator-activated receptor γ gene in mice results in lowered expression of ABCA1, ABCG1, and apoE in macrophages and reduced cholesterol efflux," *Molecular and Cellular Biology*, vol. 22, no. 8, pp. 2607–2619, 2002.

[51] D. Altshuler, J. N. Hirschhorn, M. Klannemark, et al., "The common PPARγ Pro12Ala polymorphism is associated with decreased risk of type 2 diabetes," *Nature Genetics*, vol. 26, no. 1, pp. 76–80, 2000.

[52] M. M. Swarbrick, C. M. L. Chapman, B. M. McQuillan, J. Hung, P. L. Thompson, and J. P. Beilby, "A Pro12Ala polymorphism in the human peroxisome proliferator-activated receptor-γ2 is associated with combined hyperlipidaemia in obesity," *European Journal of Endocrinology*, vol. 144, no. 3, pp. 277–282, 2001.

[53] B. A. Beamer, C.-J. Yen, R. E. Andersen, et al., "Association of the Pro12Ala variant in the peroxisome proliferator- activated receptor-γ2 gene with obesity in two Caucasian populations," *Diabetes*, vol. 47, no. 11, pp. 1806–1808, 1998.

[54] E. C. Tok, A. Aktas, D. Ertunc, E. M. Erdal, and S. Dilek, "Evaluation of glucose metabolism and reproductive hormones in polycystic ovary syndrome on the basis of peroxisome proliferator-activated receptor (PPAR)-γ2 Pro12Ala genotype," *Human Reproduction*, vol. 20, no. 6, pp. 1590–1595, 2005.

[55] A. Meirhaeghe, L. Fajas, N. Helbecque, et al., "A genetic polymorphism of the peroxisome proliferator-activated receptor γ gene influences plasma leptin levels in obese humans," *Human Molecular Genetics*, vol. 7, no. 3, pp. 435–440, 1998.

[56] A. Doney, B. Fischer, D. Frew, et al., "Haplotype analysis of the PPARγ Pro12Ala and C1431T variants reveals opposing associations with body weight," *BMC Genetics*, vol. 3, no. 1, p. 21, 2002.

[57] J. Ek, G. Andersen, S. A. Urhammer, et al., "Studies of the Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ2 (PPAR-γ2) gene in relation to insulin sensitivity among glucose tolerant caucasians," *Diabetologia*, vol. 44, no. 9, pp. 1170–1176, 2001.

[58] C. Vigouroux, L. Fajas, E. Khallouf, et al., "Human peroxisome proliferator-activated receptor-γ2: genetic mapping, identification of a variant in the coding sequence, and exclusion as the gene responsible for lipoatrophic diabetes," *Diabetes*, vol. 47, no. 3, pp. 490–492, 1998.

[59] J. Pihlajamäki, R. Miettinen, R. Valve, et al., "The Pro12Ala substitution in the peroxisome proliferator activated receptor γ 2 is associated with an insulin-sensitive phenotype in families with familial combined hyperlipidemia and in non-diabetic elderly subjects with dyslipidemia," *Atherosclerosis*, vol. 151, no. 2, pp. 567–574, 2000.

[60] A. Hamann, H. Münzberg, P. Buttron, et al., "Missense variants in the human peroxisome proliferator-activated receptor-γ2 gene in lean and obese subjects," *European Journal of Endocrinology*, vol. 141, no. 1, pp. 90–92, 1999.

[61] J. Ringel, S. Engeli, A. Distler, and A. M. Sharma, "Pro12Ala missense mutation of the peroxisome proliferator activated receptor γ and diabetes mellitus," *Biochemical and Biophysical Research Communications*, vol. 254, no. 2, pp. 450–453, 1999.

[62] Y. Mori, H. Kim-Motoyama, T. Katakura, et al., "Effect of the Pro12Ala variant of the human peroxisome proliferator-activated receptor γ2 gene on adiposity, fat distribution, and insulin sensitivity in Japanese men," *Biochemical and Biophysical Research Communications*, vol. 251, no. 1, pp. 195–198, 1998.

[63] M. Koch, K. Rett, E. Maerker, et al., "The PPARγ2 amino acid polymorphism Pro12Ala is prevalent in offspring of type II diabetic patients and is associated to increased insulin sensitivity in a subgroup of obese subjects," *Diabetologia*, vol. 42, no. 6, pp. 758–762, 1999.

[64] K. Hara, T. Okada, K. Tobe, et al., "The Pro12Ala polymorphism in PPAR γ2 may confer resistance to type 2 diabetes," *Biochemical and Biophysical Research Communications*, vol. 271, no. 1, pp. 212–216, 2000.

[65] M. Yilmaz, M. Ali Ergün, A. Karakoç, E. Yurtçu, N. Çakir, and M. Arslan, "Pro12Ala polymorphism of the peroxisome proliferator-activated receptor-γ gene in women with polycystic ovary syndrome," *Gynecological Endocrinology*, vol. 22, no. 6, pp. 336–342, 2006.

[66] S. Hahn, A. Fingerhut, U. Khomtsiv, et al., "The peroxisome proliferator activated receptor γ Pro12Ala polymorphism is associated with a lower hirsutism score and increased insulin sensitivity in women with polycystic ovary syndrome," *Clinical Endocrinology*, vol. 62, no. 5, pp. 573–579, 2005.

[67] S. Korhonen, S. Heinonen, M. Hiltunen, et al., "Polymorphism in the peroxisome proliferator-activated receptor-γ gene in women with polycystic ovary syndrome," *Human Reproduction*, vol. 18, no. 3, pp. 540–543, 2003.

[68] H. E. Xu, M. H. Lambert, V. G. Montana, et al., "Structural determinants of ligand binding selectivity between the peroxisome proliferator-activated receptors," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 24, pp. 13919–13924, 2001.

[69] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[70] A. Tsafriri, "Ovulation as a tissue remodelling process: proteolysis and cumulus expansion," *Advances in Experimental Medicine and Biology*, vol. 377, pp. 121–140, 1995.

[71] B. Dennefors, L. Hamberger, and T. Hillensjo, "Aspects concerning the role of prostaglandins for ovarian function," *Acta Obstetricia et Gynecologica Scandinavica*, vol. 113, supplement, pp. 31–41, 1983.

[72] G. Evans, M. Dobias, G. J. King, and D. T. Armstrong, "Production of prostaglandins by porcine preovulatory follicular tissues and their roles in intrafollicular function," *Biology of Reproduction*, vol. 28, no. 2, pp. 322–328, 1983.

[73] D. T. Armstrong, "Prostaglandins and follicular functions," *Journal of Reproduction and Fertility*, vol. 62, no. 1, pp. 283–291, 1981.

[74] A. V. Pontsler, A. St Hilaire, G. K. Marathe, G. A. Zimmerman, and T. M. McIntyre, "Cyclooxygenase-2 is induced in monocytes by peroxisome proliferator activated receptor γ and oxidized alkyl phospholipids from oxidized low density lipoprotein," *Journal of Biological Chemistry*, vol. 277, no. 15, pp. 13029–13036, 2002.

[75] K. Rautio, J. S. Tapanainen, A. Ruokonen, and L. C. Morin-Papunen, "Rosiglitazone treatment alleviates inflammation and improves liver function in overweight women with polycystic ovary syndrome: a randomized placebo-controlled study," *Fertility and Sterility*, vol. 87, no. 1, pp. 202–206, 2007.

[76] K. Rautio, J. S. Tapanainen, A. Ruokonen, and L. C. Morin-Papunen, "Endocrine and metabolic effects of rosiglitazone in overweight women with PCOS: a randomized placebo-controlled study," *Human Reproduction*, vol. 21, no. 6, pp. 1400–1407, 2006.

[77] A. A. Rouzi and M. S. M. Ardawi, "A randomized controlled trial of the efficacy of rosiglitazone and clomiphene citrate versus metformin and clomiphene citrate in women with clomiphene citrate-resistant polycystic ovary syndrome," *Fertility and Sterility*, vol. 85, no. 2, pp. 428–435, 2006.

[78] M. Mitkov, B. Pehlivanov, and D. Terzieva, "Metformin versus rosiglitazone in the treatment of polycystic ovary syndrome," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 126, no. 1, pp. 93–98, 2006.

[79] N. A. Cataldo, F. Abbasi, T. L. McLaughlin, et al., "Metabolic and ovarian effects of rosiglitazone treatment for 12 weeks in insulin-resistant women with polycystic ovary syndrome," *Human Reproduction*, vol. 21, no. 1, pp. 109–120, 2006.

[80] A. Lemay, S. Dodin, L. Turcot, F. Déchêne, and J.-C. Forest, "Rosiglitazone and ethinyl estradiol/cyproterone acetate as single and combined treatment of overweight women with polycystic ovary syndrome and insulin resistance," *Human Reproduction*, vol. 21, no. 1, pp. 121–128, 2006.

[81] H. M. Garmes, M. A. Tambascia, and D. E. Zantut-Wittmann, "Endocrine-metabolic effects of the treatment with pioglitazone in obese patients with polycystic ovary syndrome," *Gynecological Endocrinology*, vol. 21, no. 6, pp. 317–323, 2005.

[82] M. Yilmaz, A. Biri, A. Karakoç, et al., "The effects of rosiglitazone and metformin on insulin resistance and serum androgen levels in obese and lean patients with polycystic ovary syndrome," *Journal of Endocrinological Investigation*, vol. 28, no. 11, pp. 1003–1008, 2005.

[83] M. Yilmaz, N. Bukan, G. Ayvaz, et al., "The effects of rosiglitazone and metformin on oxidative stress and homocysteine levels in lean patients with polycystic ovary syndrome," *Human Reproduction*, vol. 20, no. 12, pp. 3333–3340, 2005.

[84] M. Yilmaz, A. Karakoç, F. B. Törüner, et al., "The effects of rosiglitazone and metformin on menstrual cyclicity and hirsutism in polycystic ovary syndrome," *Gynecological Endocrinology*, vol. 21, no. 3, pp. 154–160, 2005.

[85] I. Tarkun, B. Çetinarslan, E. Türemen, T. Şahin, Z. Cantürk, and B. Komsuoğlu, "Effect of rosiglitazone on insulin resistance, C-reactive protein and endothelial function in non-obese young women with polycystic ovary syndrome," *European Journal of Endocrinology*, vol. 153, no. 1, pp. 115–121, 2005.

[86] D. Dereli, T. Dereli, F. Bayraktar, A. G. Ozgen, and C. Yilmaz, "Endocrine and metabolic effects of rosiglitazone in non-obese women with polycystic ovary disease," *Endocrine Journal*, vol. 52, no. 3, pp. 299–308, 2005.

[87] R. V. Mehta, K. S. Patel, M. S. Coffler, et al., "Luteinizing hormone secretion is not influenced by insulin infusion in women with polycystic ovary syndrome despite improved insulin sensitivity during pioglitazone treatment," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 4, pp. 2136–2141, 2005.

[88] C. Ortega-González, S. Luna, L. Hernández, et al., "Responses of serum androgen and insulin resistance to metformin and pioglitazone in obese, insulin-resistant women with polycystic ovary syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1360–1365, 2005.

[89] V. Sepilian and M. Nagamani, "Effects of rosiglitazone in obese women with polycystic ovary syndrome and severe insulin resistance," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 1, pp. 60–65, 2005.

[90] M. F. M. Mitwally, N. K. Kuscu, and T. M. Yalcinkaya, "High ovulatory rates with use of troglitazone in clomiphene-resistant women with polycystic ovary syndrome," *Human Reproduction*, vol. 14, no. 11, pp. 2700–2703, 1999.

[91] I. Hasegawa, H. Murakawa, M. Suzuki, Y. Yamamoto, T. Kurabayashi, and K. Tanaka, "Effect of troglitazone on endocrine and ovulatory performance in women with insulin resistance-related polycystic ovary syndrome," *Fertility and Sterility*, vol. 71, no. 2, pp. 323–327, 1999.

[92] D. A. Ehrmann, D. J. Schneider, B. E. Sobel, et al., "Troglitazone improves defects in insulin action, insulin secretion, ovarian steroidogenesis, and fibrinolysis in women with polycystic ovary syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 7, pp. 2108–2116, 1997.

[93] R. Azziz, D. Ehrmann, R. S. Legro, et al., "Troglitazone improves ovulation and hirsutism in the polycystic ovary syndrome: a multicenter, double blind, placebo-controlled trial,"

Journal of Clinical Endocrinology and Metabolism, vol. 86, no. 4, pp. 1626–1632, 2001.

[94] F. Picard and J. Auwerx, "PPARγ and glucose homeostasis," *Annual Review of Nutrition*, vol. 22, pp. 167–197, 2002.

[95] N. Brettenthaler, C. De Geyter, P. R. Huber, and U. Keller, "Effect of the insulin sensitizer pioglitazone on insulin resistance, hyperandrogenism, and ovulatory dysfunction in women with polycystic ovary syndrome," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 8, pp. 3835–3840, 2004.

[96] S. H. Belli, M. N. Graffigna, A. Oneto, P. Otero, L. Schurman, and O. A. Levalle, "Effect of rosiglitazone on insulin resistance, growth factors, and reproductive disturbances in women with polycystic ovary syndrome," *Fertility and Sterility*, vol. 81, no. 3, pp. 624–629, 2004.

[97] A. Shobokshi and M. Shaarawy, "Correction of insulin resistance and hyperandrogenism in polycystic ovary syndrome by combined rosiglitazone and clomiphene citrate therapy," *Journal of the Society for Gynecologic Investigation*, vol. 10, no. 2, pp. 99–104, 2003.

[98] D. Seto-Young, M. Paliou, J. Schlosser, et al., "Direct thiazolidinedione action in the human ovary: insulin-independent and insulin-sensitizing effects on steroidogenesis and insulin-like growth factor binding protein-1 production," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 11, pp. 6099–6105, 2005.

[99] S. Gasic, M. Nagamani, A. Green, and R. J. Urban, "Troglitazone is a competitive inhibitor of 3β-hydroxysteroid dehydrogenase enzyme in the ovary," *American Journal of Obstetrics and Gynecology*, vol. 184, no. 4, pp. 575–579, 2001.

[100] M. S. Coffler, K. Patel, M. H. Dahan, R. Y. Yoo, P. J. Malcom, and R. J. Chang, "Enhanced granulosa cell responsiveness to follicle-stimulating hormone during insulin infusion in women with polycystic ovary syndrome treated with pioglitazone," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 12, pp. 5624–5631, 2003.

[101] C. E. Minge, B. D. Bennett, M. Lane, R. J. Norman, and R. L. Robker, "Obesity-induced female infertility arises from impaired oocyte developmental competence and can be reversed by peri-ovulatory rosiglitazone treatment," in *Proceedings of the 10th International Congress on Obesity*, Sydney, Australia, 2006.

[102] W. L. Miller, "Steroidogenic acute regulatory protein (StAR), a novel mitochondrial cholesterol transporter," *Biochimica et Biophysica Acta—Molecular and Cell Biology of Lipids*, vol. 1771, no. 6, pp. 663–676, 2007.

[103] D. Seto-Young, D. Avtanski, M. Strizhevsky, et al., "Interactions among peroxisome proliferator activated receptor-γ, insulin signaling pathways, and steroidogenic acute regulatory protein in human ovarian cells," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 6, pp. 2232–2239, 2007.

[104] W. C. Duncan, G. M. Cowen, and P. J. Illingworth, "Steroidogenic enzyme expression in human corpora lutea in the presence and absence of exogenous human chorionic gonadotrophin (HCG)," *Molecular Human Reproduction*, vol. 5, no. 4, pp. 291–298, 1999.

[105] K. J. Doody, M. C. Lorence, J. I. Mason, and E. R. Simpson, "Expression of messenger ribonucleic acid species encoding steroidogenic enzymes in human follicles and corpora lutea throughout the menstrual cycle," *Journal of Clinical Endocrinology and Metabolism*, vol. 70, no. 4, pp. 1041–1045, 1990.

[106] W. Arlt, R. J. Auchus, and W. L. Miller, "Thiazolidinediones but not metformin directly inhibit the steroidogenic enzymes P450c17 and 3β-hydroxysteroid dehydrogenase," *Journal of Biological Chemistry*, vol. 276, no. 20, pp. 16767–16771, 2001.

[107] P. Kempná, G. Hofer, P. E. Mullis, and C. E. Flück, "Pioglitazone inhibits androgen production in NCI-H295R cells by regulating gene expression of CYP17 and HSD3B2," *Molecular Pharmacology*, vol. 71, no. 3, pp. 787–798, 2007.

[108] J. D. Veldhuis, G. Zhang, and J. C. Garmey, "Troglitazone, an insulin-sensitizing thiazolidinedione, represses combined stimulation by LH and insulin of de novo androgen biosynthesis by thecal cells in vitro," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 3, pp. 1129–1133, 2002.

[109] M. P. Steinkampf, C. R. Mendelson, and E. R. Simpson, "Regulation by follicle-stimulating hormone of the synthesis of aromatase cytochrome P-450 in human granulosa cells," *Molecular Endocrinology*, vol. 1, no. 7, pp. 465–471, 1987.

[110] J. S. Krasnow, G. J. Hickey, and J. S. Richards, "Regulation of aromatase mRNA and estradiol biosynthesis in rat ovarian granulosa and luteal cells by prolactin," *Molecular Endocrinology*, vol. 4, no. 1, pp. 13–21, 1990.

[111] T. Yanase, Y.-M. Mu, Y. Nishi, et al., "Regulation of aromatase by nuclear receptors," *Journal of Steroid Biochemistry and Molecular Biology*, vol. 79, no. 1–5, pp. 187–192, 2001.

[112] Y.-M. Mu, T. Yanase, Y. Nishi, R. Takayanagi, K. Goto, and H. Nawata, "Combined treatment with specific ligands for PPARγ:RXR nuclear receptor system markedly inhibits the expression of cytochrome P450arom in human granulosa cancer cells," *Molecular and Cellular Endocrinology*, vol. 181, no. 1–2, pp. 239–248, 2001.

[113] W. Fan, T. Yanase, H. Morinaga, et al., "Activation of peroxisome proliferator-activated receptor-γ and retinoid X receptor inhibits aromatase transcription via nuclear factor-κB," *Endocrinology*, vol. 146, no. 1, pp. 85–92, 2005.

[114] J.-P. Baillargeon, D. J. Jakubowicz, M. J. Iuorno, S. Jakubowicz, and J. E. Nestler, "Effects of metformin and rosiglitazone, alone and in combination, in nonobese women with polycystic ovary syndrome and normal indices of insulin sensitivity," *Fertility and Sterility*, vol. 82, no. 4, pp. 893–902, 2004.

[115] D. Romualdi, M. Guido, M. Ciampelli, et al., "Selective effects of pioglitazone on insulin and androgen abnormalities in normo- and hyperinsulinaemic obese patients with polycystic ovary syndrome," *Human Reproduction*, vol. 18, no. 6, pp. 1210–1218, 2003.

[116] T. Lovekamp-Swan, A. M. Jetten, and B. J. Davis, "Dual activation of PPARα and PPARγ by mono-(2-ethylhexyl) phthalate in rat ovarian granulosa cells," *Molecular and Cellular Endocrinology*, vol. 201, no. 1–2, pp. 133–141, 2003.

[117] A. Rubenstrunk, R. Hanf, D. W. Hum, J.-C. Fruchart, and B. Staels, "Safety issues and prospects for future generations of PPAR modulators," *Biochimica et Biophysica Acta—Molecular and Cell Biology of Lipids*, vol. 1771, no. 8, pp. 1065–1081, 2007.

[118] A. V. Schwartz, D. E. Sellmeyer, E. Vittinghoff, et al., "Thiazolidinedione use and bone loss in older diabetic adults," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 9, pp. 3349–3354, 2006.

[119] D. B. Klinkner, H. J. Lim, E. Y. Strawn Jr., K. T. Oldham, and T. L. Sander, "An in vivo murine model of rosiglitazone use in pregnancy," *Fertility and Sterility*, vol. 86, no. 4, pp. 1074–1079, 2006.

[120] N. A. Cataldo, F. Abbasi, T. L. McLaughlin, C. Lamendola, and G. M. Reaven, "Improvement in insulin sensitivity followed by ovulation and pregnancy in a woman

with polycystic ovary syndrome who was treated with rosiglitazone," *Fertility and Sterility*, vol. 76, no. 5, pp. 1057–1059, 2001.

[121] L. Y.-S. Chan, J. H.-K. Yeung, and T. K. Lau, "Placental transfer of rosiglitazone in the first trimester of human pregnancy," *Fertility and Sterility*, vol. 83, no. 4, pp. 955–958, 2005.

[122] A. Dunaif, "Insulin action in the polycystic ovary syndrome," *Endocrinology and Metabolism Clinics of North America*, vol. 28, no. 2, pp. 341–359, 1999.

[123] L. Poretsky, J. Clemons, and K. Bogovich, "Hyperinsulinemia and human chorionic gonadotropin synergistically promote the growth of ovarian follicular cysts in rats," *Metabolism*, vol. 41, no. 8, pp. 903–910, 1992.

[124] N. K. Kuşcu, F. Koyuncu, K. Özbilgin, S. Inan, I. Tuğlu, and Ö. Karaer, "Insulin: does it induce follicular arrest in the rat ovary?" *Gynecological Endocrinology*, vol. 16, no. 5, pp. 361–364, 2002.

[125] S. Hashimoto, N. Minami, M. Yamada, and H. Imai, "Excessive concentration of glucose during in vitro maturation impairs the developmental competence of bovine oocytes after in vitro fertilization: relevance to intracellular reactive oxygen species and glutathione contents," *Molecular Reproduction and Development*, vol. 56, no. 4, pp. 520–526, 2000.

[126] S. A. Colton, G. M. Pieper, and S. M. Downs, "Altered meiotic regulation in oocytes from diabetic mice," *Biology of Reproduction*, vol. 67, no. 1, pp. 220–231, 2002.

[127] J. Rieusset, J. Auwerx, and H. Vidal, "Regulation of gene expression by activation of the peroxisome proliferator-activated receptor γ with rosiglitazone (BRL 49653) in human adipocytes," *Biochemical and Biophysical Research Communications*, vol. 265, no. 1, pp. 265–271, 1999.

[128] U. Smith, S. Gogg, A. Johansson, T. Olausson, V. Rotter, and B. Svalstedt, "Thiazolidinediones (PPARγ agonists) but not PPARα agonists increase IRS-2 gene expression in 3T3-L1 and human adipocytes," *FASEB Journal*, vol. 15, no. 1, pp. 215–220, 2001.

[129] T. P. Ciaraldi, A. P. S. Kong, N. V. Chu, et al., "Regulation of glucose transport and insulin signaling by troglitazone or metformin in adipose tissue of type 2 diabetic subjects," *Diabetes*, vol. 51, no. 1, pp. 30–36, 2002.

[130] L. M. Furtado, V. Poon, and A. Klip, "GLUT4 activation: thoughts on possible mechanisms," *Acta Physiologica Scandinavica*, vol. 178, no. 4, pp. 287–296, 2003.

[131] N. Khandoudi, P. Delerive, I. Berrebi-Bertrand, R. E. Buckingham, B. Staels, and A. Bril, "Rosiglitazone, a peroxisome proliferator-activated receptor-γ, inhibits the Jun NH2-terminal kinase/activating protein 1 pathway and protects the heart from ischemia/reperfusion injury," *Diabetes*, vol. 51, no. 5, pp. 1507–1514, 2002.

[132] G. Jiang, Q. Dallas-Yang, S. Biswas, Z. Li, and B. B. Zhang, "Rosiglitazone, an agonist of peroxisome-proliferator-activated receptor γ (PPARγ), decreases inhibitory serine phosphorylation of IRS1 in vitro and in vivo," *Biochemical Journal*, vol. 377, no. 2, pp. 339–346, 2004.

[133] G. Jiang and B. B. Zhang, "Modulation of insulin signalling by insulin sensitizers," *Biochemical Society Transactions*, vol. 33, no. 2, pp. 358–361, 2005.

[134] L. Guo and R. Tabrizchi, "Peroxisome proliferator-activated receptor gamma as a drug target in the pathogenesis of insulin resistance," *Pharmacology and Therapeutics*, vol. 111, no. 1, pp. 145–173, 2006.

[135] S.-Y. Ku, S. D. Kim, B. C. Jee, et al., "Clinical efficacy of body mass index as predictor of in vitro fertilization and embryo transfer outcomes," *Journal of Korean Medical Science*, vol. 21, no. 2, pp. 300–303, 2006.

[136] A. Bongain, V. Isnard, and J.-Y. Gillet, "Obesity in obstetrics and gynaecology," *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 77, no. 2, pp. 217–228, 1998.

[137] Y. Linné, "Effects of obesity on women's reproduction and complications during pregnancy," *Obesity Reviews*, vol. 5, no. 3, pp. 137–143, 2004.

[138] G. A. Bray, "Obesity and reproduction," *Human Reproduction*, vol. 12, no. 1, pp. 26–32, 1997.

[139] J. X. Wang, M. Davies, and R. J. Norman, "Body mass and probability of pregnancy during assisted reproduction treatment: retrospective study," *British Medical Journal*, vol. 321, no. 7272, pp. 1320–1321, 2000.

[140] R. Pasquali, C. Pelusi, S. Genghini, M. Cacciari, and A. Gambineri, "Obesity and reproductive disorders in women," *Human Reproduction Update*, vol. 9, no. 4, pp. 359–372, 2003.

[141] B. M. Zaadstra, J. C. Seidell, P. A. H. Van Noord, et al., "Fat and female fecundity: prospective study of effect of body fat distribution on conception rates," *British Medical Journal*, vol. 306, no. 6876, pp. 484–487, 1993.

[142] A. Fukuhara, M. Matsuda, M. Nishizawa, et al., "Visfatin: a protein secreted by visceral fat that mimics the effects of insulin," *Science*, vol. 307, no. 5708, pp. 426–430, 2005.

[143] T. E. Graham, Q. Yang, M. Blüher, et al., "Retinol-binding protein 4 and insulin resistance in lean, obese, and diabetic subjects," *New England Journal of Medicine*, vol. 354, no. 24, pp. 2552–2563, 2006.

[144] B. K. Tan, J. Chen, J. E. Digby, S. D. Keay, C. R. Kennedy, and H. S. Randeva, "Increased visfatin messenger ribonucleic acid and protein levels in adipose tissue and adipocytes in women with polycystic ovary syndrome: parallel increase in plasma visfatin," *Journal of Clinical Endocrinology and Metabolism*, vol. 91, no. 12, pp. 5022–5028, 2006.

[145] B. K. Tan, J. Chen, H. Lehnert, R. Kennedy, and H. S. Randeva, "Raised serum, adipocyte, and adipose tissue retinol-binding protein 4 in overweight women with polycystic ovary syndrome: effects of gonadal and adrenal steroids," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 7, pp. 2764–2772, 2007.

[146] M. Iwaki, M. Matsuda, N. Maeda, et al., "Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors," *Diabetes*, vol. 52, no. 7, pp. 1655–1663, 2003.

[147] P. De Vos, A.-M. Lefebvre, S. G. Miller, et al., "Thiazolidinediones repress ob gene expression in rodents via activation of peroxisome proliferator-activated receptor γ," *Journal of Clinical Investigation*, vol. 98, no. 4, pp. 1004–1009, 1996.

[148] D. Sinha, S. Addya, E. Murer, and G. Boden, "15-Deoxy-$\Delta^{12,14}$ prostaglandin J2: a putative endogenous promoter of adipogenesis suppresses the *ob* gene," *Metabolism*, vol. 48, no. 6, pp. 786–791, 1999.

[149] C. B. Kallen and M. A. Lazar, "Antidiabetic thiazolidinediones inhibit leptin (*ob*) gene expression in 3T3-L1 adipocytes," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 93, no. 12, pp. 5793–5796, 1996.

[150] Y. Miyazaki, A. Mahankali, E. Wajcberg, M. Bajaj, L. J. Mandarino, and R. A. DeFronzo, "Effect of pioglitazone on circulating adipocytokine levels and insulin sensitivity in type 2 diabetic patients," *Journal of Clinical Endocrinology and Metabolism*, vol. 89, no. 9, pp. 4312–4319, 2004.

[151] C. Otto, B. Otto, B. Göke, et al., "Increase in adiponectin levels during pioglitazone therapy in relation to glucose control, insulin resistance as well as ghrelin and resistin levels," *Journal of Endocrinological Investigation*, vol. 29, no. 3, pp. 231–236, 2006.

[152] L. J. Spicer and C. C. Francisco, "The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function," *Endocrinology*, vol. 138, no. 8, pp. 3374–3379, 1997.

[153] C. Karlsson, K. Lindell, E. Svensson, et al., "Expression of functional leptin receptors in the human ovary," *Journal of Clinical Endocrinology and Metabolism*, vol. 82, no. 12, pp. 4144–4148, 1997.

[154] N. K. Ryan, K. H. Van der Hoek, S. A. Robertson, and R. J. Norman, "Leptin and leptin receptor expression in the rat ovary," *Endocrinology*, vol. 144, no. 11, pp. 5006–5013, 2003.

[155] R. J. Zachow and D. A. Magoffin, "Direct intraovarian effects of leptin: impairment of the synergistic action of insulin-like growth factor-I on follicle-stimulating hormone- dependent estradiol-17β production by rat ovarian granulosa cells," *Endocrinology*, vol. 138, no. 2, pp. 847–850, 1997.

[156] N. K. Ryan, C. M. Woodhouse, K. H. Van der Hoek, R. B. Gilchrist, D. T. Armstrong, and R. J. Norman, "Expression of leptin and its receptor in the murine ovary: possible role in the regulation of oocyte maturation," *Biology of Reproduction*, vol. 66, no. 5, pp. 1548–1554, 2002.

[157] J. E. Swain, R. L. Dunn, D. McConnell, J. Gonzalez-Martinez, and G. D. Smith, "Direct effects of leptin on mouse reproductive function: regulation of follicular, oocyte, and embryo development," *Biology of Reproduction*, vol. 71, no. 5, pp. 1446–1452, 2004.

[158] E. Lord, S. Ledoux, B. D. Murphy, D. Beaudry, and M. F. Palin, "Expression of adiponectin and its receptors in swine," *Journal of Animal Science*, vol. 83, no. 3, pp. 565–578, 2005.

[159] S. Ledoux, D. B. Campos, F. L. Lopes, M. Dobias-Goff, M.-F. Palin, and B. D. Murphy, "Adiponectin induces periovulatory changes in ovarian follicular cells," *Endocrinology*, vol. 147, no. 11, pp. 5178–5186, 2006.

[160] P. Tontonoz, E. Hu, J. Devine, E. G. Beale, and B. M. Spiegelman, "PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene," *Molecular and Cellular Biology*, vol. 15, no. 1, pp. 351–357, 1995.

[161] K. Schoonjans, J. Peinado-Onsurbe, A.-M. Lefebvre, et al., "PPARα and PPARγ activators direct a distinct tissue-specific transcriptional response via a PPRE in the lipoprotein lipase gene," *EMBO Journal*, vol. 15, no. 19, pp. 5336–5348, 1996.

[162] S. R. Ross, R. A. Graves, A. Greenstein, et al., "A fat-specific enhancer is the primary determinant of gene expression for adipocyte P2 in vivo," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 87, no. 24, pp. 9590–9594, 1990.

[163] M. Watanabe, K. Inukai, H. Katagiri, T. Awata, Y. Oka, and S. Katayama, "Regulation of PPARγ transcriptional activity in 3T3-L1 adipocytes," *Biochemical and Biophysical Research Communications*, vol. 300, no. 2, pp. 429–436, 2003.

[164] P. Tontonoz, L. Nagy, J. G. A. Alvarez, V. A. Thomazy, and R. M. Evans, "PPARγ promotes monocyte/macrophage differentiation and uptake of oxidized LDL," *Cell*, vol. 93, no. 2, pp. 241–252, 1998.

[165] G. Chinetti, F. G. Gbaguidi, S. Griglio, et al., "CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors," *Circulation*, vol. 101, no. 20, pp. 2411–2417, 2000.

[166] J. J. Petrik, P. A. Gentry, J.-J. Feige, and J. LaMarre, "Expression and localization of thrombospondin-1 and -2 and their cell-surface receptor, CD36, during rat follicular development and formation of the corpus luteum," *Biology of Reproduction*, vol. 67, no. 5, pp. 1522–1531, 2002.

[167] J. Greenaway, P. A. Gentry, J.-J. Feige, J. LaMarre, and J. J. Petrik, "Thrombospondin and vascular endothelial growth factor are cyclically expressed in an inverse pattern during bovine ovarian follicle development," *Biology of Reproduction*, vol. 72, no. 5, pp. 1071–1078, 2005.

[168] J. E. Murphy-Ullrich, "The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state?" *Journal of Clinical Investigation*, vol. 107, no. 7, pp. 785–790, 2001.

[169] S. Goicoechea, A. W. Orr, M. A. Pallero, P. Eggleton, and J. E. Murphy-Ullrich, "Thrombospondin mediates focal adhesion disassembly through interactions with cell surface calreticulin," *Journal of Biological Chemistry*, vol. 275, no. 46, pp. 36358–36368, 2000.

[170] L. Nagy, P. Tontonoz, J. G. A. Alvarez, H. Chen, and R. M. Evans, "Oxidized LDL regulates macrophage gene expression through ligand activation of PPARγ," *Cell*, vol. 93, no. 2, pp. 229–240, 1998.

[171] M. Ricote, J. T. Huang, J. S. Welch, and C. K. Glass, "The peroxisome proliferator-activated receptorγ (PPARγ) as a regulator of monocyte/macrophage function," *Journal of Leukocyte Biology*, vol. 66, no. 5, pp. 733–739, 1999.

[172] P.-A. Svensson, M. S. C. Johnson, C. Ling, L. M. S. Carlsson, H. Billig, and B. Carlsson, "Scavenger receptor class B type I in the rat ovary: possible role in high density lipoprotein cholesterol uptake and in the recognition of apoptotic granulosa cells," *Endocrinology*, vol. 140, no. 6, pp. 2494–2500, 1999.

[173] E. Reaven, A. Nomoto, S. Leers-Sucheta, R. Temel, D. L. Williams, and S. Azhar, "Expression and microvillar localization of scavenger receptor, class B, type I (a high density lipoprotein receptor) in luteinized and hormone- desensitized rat ovarian models," *Endocrinology*, vol. 139, no. 6, pp. 2847–2856, 1998.

[174] M. Ricote, J. Huang, L. Fajas, et al., "Expression of the peroxisome proliferator-activated receptor γ (PPARγ) in human atherosclerosis and regulation in macrophages by colony stimulating factors and oxidized low density lipoprotein," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 13, pp. 7614–7619, 1998.

[175] M. Ricote, A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass, "The peroxisome proliferator-activated receptor-γ is a negative regulator of macrophage activation," *Nature*, vol. 391, no. 6662, pp. 79–82, 1998.

[176] R. B. Clark, D. Bishop-Bailey, T. Estrada-Hernandez, T. Hla, L. Puddington, and S. J. Padula, "The nuclear receptor PPARγ and immunoregulation: PPARγ mediates inhibition of helper T cell responses," *Journal of Immunology*, vol. 164, no. 3, pp. 1364–1371, 2000.

[177] Y. Azuma, M. Shinohara, P.-L. Wang, and K. Ohura, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits IL-10 and IL-12 production by macrophages," *Biochemical and Biophysical Research Communications*, vol. 283, no. 2, pp. 344–346, 2001.

[178] P. Wang, P. O. Anderson, S. Chen, K. M. Paulsson, H.-O. Sjögren, and S. Li, "Inhibition of the transcription factors AP-1 and NF-κB in CD4 T cells by peroxisome proliferator-activated receptor γ ligands," *International Immunopharmacology*, vol. 1, no. 4, pp. 803–812, 2001.

[179] D. G. Alleva, E. B. Johnson, F. M. Lio, S. A. Boehme, P. J. Conlon, and P. D. Crowe, "Regulation of murine macrophage proinflammatory and anti-inflammatory cytokines by ligands for peroxisome proliferator-activated receptor-γ: counter-regulatory activity by IFN-γ," *Journal of Leukocyte Biology*, vol. 71, no. 4, pp. 677–685, 2002.

[180] M. Okada, S. F. Yan, and D. J. Pinsky, "Peroxisome proliferator-activated receptor-γ (PPAR-γ) activation suppresses ischemic induction of Egr-1 and its inflammatory gene targets," *FASEB Journal*, vol. 16, no. 14, pp. 1861–1868, 2002.

[181] B. Hinz, K. Brune, and A. Pahl, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits the expression of proinflammatory genes in human blood monocytes via a PPAR-γ-independent mechanism," *Biochemical and Biophysical Research Communications*, vol. 302, no. 2, pp. 415–420, 2003.

[182] K. Asada, S. Sasaki, T. Suda, K. Chida, and H. Nakamura, "Antiinflammatory roles of peroxisome proliferator-activated receptor γ in human alveolar macrophages," *American Journal of Respiratory and Critical Care Medicine*, vol. 169, no. 2, pp. 195–200, 2004.

[183] G. Chinetti, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation," *Inflammation Research*, vol. 49, no. 10, pp. 497–505, 2000.

[184] P. Delerive, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors in inflammation control," *Journal of Endocrinology*, vol. 169, no. 3, pp. 453–459, 2001.

[185] P. Henson, "Suppression of macrophage inflammatory responses by PPARs," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 11, pp. 6295–6296, 2003.

[186] B. Zahorska-Markiewicz, J. Janowska, M. Olszanecka-Glinianowicz, and A. Zurakowski, "Serum concentrations of TNF-α and soluble TNF-α receptors in obesity," *International Journal of Obesity*, vol. 24, no. 11, pp. 1392–1395, 2000.

[187] M. Olszanecka-Glinianowicz, B. Zahorska-Markiewicz, J. Janowska, and A. Zurakowski, "Serum concentrations of nitric oxide, tumor necrosis factor (TNF)-α and TNF soluble receptors in women with overweight and obesity," *Metabolism: Clinical and Experimental*, vol. 53, no. 10, pp. 1268–1273, 2004.

[188] G. S. Hotamisligil, N. S. Shargill, and B. M. Spiegelman, "Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance," *Science*, vol. 259, no. 5091, pp. 87–91, 1993.

[189] J. M. Stephens, J. Lee, and P. F. Pilch, "Tumor necrosis factor-α-induced insulin resistance in 3T3-L1 adipocytes is accompanied by a loss of insulin receptor substrate-1 and GLUT4 expression without a loss of insulin receptor-mediated signal transduction," *Journal of Biological Chemistry*, vol. 272, no. 2, pp. 971–976, 1997.

[190] G. Kroder, B. Bossenmaier, M. Kellerer, et al., "Tumor necrosis factor-α- and hyperglycemia-induced insulin resistance: evidence for different mechanisms and different effects on insulin signaling," *Journal of Clinical Investigation*, vol. 97, no. 6, pp. 1471–1477, 1996.

[191] J. S. Yudkin, M. Kumari, S. E. Humphries, and V. Mohamed-Ali, "Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link?" *Atherosclerosis*, vol. 148, no. 2, pp. 209–214, 2000.

[192] M. Olszanecka-Glinianowicz, M. Banaś, B. Zahorska-Markiewicz, et al., "Is the polycystic ovary syndrome associated with chronic inflammation per se?" *European Journal of Obstetrics Gynecology and Reproductive Biology*, vol. 133, no. 2, pp. 197–202, 2007.

[193] Y.-F. Zhang, Y.-S. Yang, J. Hong, et al., "Elevated serum levels of interleukin-18 are associated with insulin resistance in women with polycystic ovary syndrome," *Endocrine*, vol. 29, no. 3, pp. 419–423, 2006.

[194] P. O. Szapary, L. T. Bloedon, F. F. Samaha, et al., "Effects of pioglitazone on lipoproteins, inflammatory markers, and adipokines in nondiabetic patients with metabolic syndrome," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 1, pp. 182–188, 2006.

[195] F. F. Samaha, P. O. Szapary, N. Iqbal, et al., "Effects of rosiglitazone on lipids, adipokines, and inflammatory markers in nondiabetic patients with low high-density lipoprotein cholesterol and metabolic syndrome," *Arteriosclerosis, Thrombosis, and Vascular Biology*, vol. 26, no. 3, pp. 624–630, 2006.

[196] K. Esposito, M. Ciotola, D. Carleo, et al., "Effect of rosiglitazone on endothelial function and inflammatory markers in patients with the metabolic syndrome," *Diabetes Care*, vol. 29, no. 5, pp. 1071–1076, 2006.

[197] T.-D. Wang, W.-J. Chen, J.-W. Lin, M.-F. Chen, and Y.-T. Lee, "Effects of Rosiglitazone on endothelial function, C-reactive protein, and components of the metabolic syndrome in nondiabetic patients with the metabolic syndrome," *American Journal of Cardiology*, vol. 93, no. 3, pp. 362–365, 2004.

[198] T.-D. Wang, W.-J. Chen, W.-C. Cheng, J.-W. Lin, M.-F. Chen, and Y.-T. Lee, "Relation of improvement in endothelium-dependent flow-mediated vasodilation after rosiglitazone to changes in asymmetric dimethylarginine, endothelin-1, and C-reactive protein in nondiabetic patients with the metabolic syndrome," *American Journal of Cardiology*, vol. 98, no. 8, pp. 1057–1062, 2006.

[199] P. Hellberg, P. Thomsen, P. O. Janson, and M. Brannstrom, "Leukocyte supplementation increases the luteinizing hormone-induced ovulation rate in the in vitro-perfused rat ovary," *Biology of Reproduction*, vol. 44, no. 5, pp. 791–797, 1991.

[200] M. Brannstrom, G. Mayrhofer, and S. A. Robertson, "Localization of leukocyte subsets in the rat ovary during the periovulatory period," *Biology of Reproduction*, vol. 48, no. 2, pp. 277–286, 1993.

[201] K. H. Van der Hoek, S. Maddocks, C. M. Woodhouse, N. Van Rooijen, S. A. Robertson, and R. J. Norman, "Intrabursal injection of clodronate liposomes causes macrophage depletion and inhibits ovulation in the mouse ovary," *Biology of Reproduction*, vol. 62, no. 4, pp. 1059–1066, 2000.

[202] P. E. Cohen, K. Nishimura, L. Zhu, and J. W. Pollard, "Macrophages: important accessory cells for reproductive function," *Journal of Leukocyte Biology*, vol. 66, no. 5, pp. 765–772, 1999.

[203] M. Brännström and P. O. Janson, "The biochemistry of ovulation," in *Ovarian Endocrinology*, pp. 133–166, Blackwell Science, Oxford, UK, 1991.

[204] R. Takaya, T. Fukaya, H. Sasano, T. Suzuki, M. Tamura, and A. Yajima, "Macrophages in normal cycling human ovaries; immunohistochemical localization and characterization," *Human Reproduction*, vol. 12, no. 7, pp. 1508–1512, 1997.

[205] E. Y. Anteby, A. Hurwitz, O. Korach, et al., "Human follicular nitric oxide pathway: relationship to follicular size, oestradiol concentrations and ovarian blood flow," *Human Reproduction*, vol. 11, no. 9, pp. 1947–1951, 1996.

[206] C. Battaglia, M. Salvatori, N. Maxia, F. Petraglia, F. Facchinetti, and A. Volpe, "Adjuvant L-arginine treatment for in-vitro fertilization in poor responder patients," *Human Reproduction*, vol. 14, no. 7, pp. 1690–1697, 1999.

[207] D. G. Lemay and D. H. Hwang, "Genome-wide identification of peroxisome proliferator response elements using integrated computational genomics," *Journal of Lipid Research*, vol. 47, no. 7, pp. 1583–1587, 2006.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 176275, 9 pages
doi:10.1155/2008/176275

Review Article

Role of the Peroxisome Proliferator-Activated Receptors, Adenosine Monophosphate-Activated Kinase, and Adiponectin in the Ovary

Joëlle Dupont, Christine Chabrolle, Christelle Ramé, Lucie Tosca, and Stéphanie Coyral-Castel

Unité de Physiologie de la Reproduction et des Comportements, Institut National de la Recherche Agronomique, 37380 Nouzilly, France

Correspondence should be addressed to Joëlle Dupont, jdupont@tours.inra.fr

Received 1 June 2007; Accepted 19 July 2007

Recommended by Pascal Froment

The mechanisms controlling the interaction between energy balance and reproduction are the subject of intensive investigations. The integrated control of these systems is probably a multifaceted phenomenon involving an array of signals governing energy homeostasis, metabolism, and fertility. Two fuel sensors, PPARs, a superfamily of nuclear receptors and the kinase AMPK, integrate energy control and lipid and glucose homeostasis. Adiponectin, one of the adipocyte-derived factors mediate its actions through the AMPK or PPARs pathway. These three molecules are expressed in the ovary, raising questions about the biological actions of fuel sensors in fertility and the use of these molecules to treat fertility problems. This review will highlight the expression and putative role of PPARs, AMPK, and adiponectin in the ovary, particularly during folliculogenesis, steroidogenesis, and oocyte maturation.

1. INTRODUCTION

The levels of various molecules, including metabolites (glucose, fatty acids, amino acids) and hormones (adiponectin, insulin, leptin, ghrelin, etc.), are modulated by nutrition and energy supply. Most of these molecules are known to be directly involved, through a fuel sensor, in the regulation of fertility at each level of the hypothalamo-pituitary-gonad axis (for review see [1, 2]). For example, mice lacking insulin-signalling pathway components, such as insulin receptor substrate 2 (IRS-2) or insulin receptor, display female and male infertility [3, 4].

In humans, a close link between energy status and reproductive function has been found in some diseases. Polycystic ovary syndrome (PCOS), which is frequently associated with insulin resistance, affects 5 to 10% of women of reproductive age [5]. Women with PCOS present with ovulation problems, which may be associated with infertility. The treatment of PCOS patients with insulin-sensitising agents of various drug families, such as thiazolidinediones (TZDs) or metformin (a derivative of biguanide), restores the menstrual cycle [6] and increases ovulation (by improving follicular growth), fertilization, and pregnancy rates [7]. TZDs bind to the nuclear peroxisome proliferator-activated receptor gamma (PPARγ) and metformin activates the AMP-activated protein kinase (AMPK) pathway [8, 9]. In women with PCOS, plasma adiponectin is also significantly decreased independently of obesity [10]. Adiponectin plasma levels seem to be related to TZDs or Metformin treatment. Adiponectin is an adipokine known to increase sensitivity to insulin and vasodilatation (for review [11]). Adiponectin could also be involved in the regulations of some reproductive functions [12, 13]. In mammals, and particularly in cattle, dietary fats also influence reproductive function. For example, fatty acid supplementation in the diet increases the total number of follicles and stimulates growth of the preovulatory follicle [14]. In cows, the availability of fatty acid precursors is coupled with an increase in sexual steroid levels and eicosanoid secretion, potentially affecting ovarian and uterine function and embryo implantation [15]. These phenomena may involve several hormones including insulin, IGFs, leptin, adiponectin, and some factors such as PPARs

and AMPK. Indeed, these molecules are known to play a role in energy control and lipid metabolism. They may hypothetically play a role as fuel sensors in reproductive compartments, providing the cells with information about energy status. However, how metformin and TZDs influence ovarian function is still under investigation. The functions of PPARs, AMPK, and adiponectin in the ovary also remain unclear. In this review, we will describe the expression and potential implications of these fuel sensors in the ovary.

2. PPARs AND AMPK STRUCTURES AND IMPLICATIONS

The PPAR family (α, β/δ and γ) integrates energy control with lipid and glucose metabolism and affects insulin sensitivity [16]. Like PPARs, AMPK plays a key role in regulating lipid and glucose metabolism in response to metabolic stress and energy demand [17]. AMPK acts at various steps and plays a central role in controlling fatty acid, triglyceride, and cholesterol synthesis, and the oxidation of fatty acids, through direct phosphorylation and control over gene transcription [17].

PPARs and AMPK have similar effects and close links have been found between these molecules. Indeed, it is generally assumed that TZDs activate PPARγ and AMPK independently [18–20]. The inhibition of AMPK expression by siRNA abolishes the inhibitory effects of rosiglitazone and 15d-PGJ_2 (two PPARγ ligands, see below) on iNOS expression and activity [21]. The mitochondria may house a pathway common to PPARγ and AMPK. Indeed, both metformin and TZDs cause a rapid increase in cellular ADP:ATP ratio, probably by inhibiting the respiratory chain, leading to the phosphorylation and activation of AMPK [22]. PPARs and AMPK also participate in the molecular action of adiponectin, an adipocytokine involved in the insulin sensitivity of tissues [7].

2.1. Structure and mechanisms of action of PPARs

The PPARs are transcription factors that share a common structure with steroid hormone receptors: the N-terminal A/B domain responsible for ligand-independent transactivation function, the C domain containing the DNA-binding domain, the D domain involved in the receptor dimerization, and the C-terminal E/F domain containing the ligand binding domain (for review [23]). The members of the nuclear PPAR (α, β/δ, and γ) family bind to specific regions of DNA in heterodimers with the retinoid X receptors (RXRs) [24]. These DNA sequences are known as PPREs (peroxisome proliferator response elements). The transcription is activated subsequent to heterodimerisation of PPAR and retinoid receptors (RXR). Furthermore, PPARs are able to indirectly regulate gene expression through transrepression mechanisms by linking some cofactors (reviewed in [23]). In this review, we focus on the PPARα and PPARγ isoforms.

The stimulation of PPARγ by TZDs modifies the transcription and/or the activity of several key regulators of energy homeostasis, including several glucose regulators (glucose transporters, insulin receptor, IRS, etc.), which it stimulates (for review see [25, 26]). PPARs regulate the transcription of a number of target genes involved in ovarian functions such as steroidogenesis, ovulation, oocyte maturation, and maintenance of the corpus luteum (cyclooxygenase-2 (COX-2), nitric oxide synthase (NOS), several proteases, including matrix metalloprotease-9, plasminogen activator, and vascular endothelial growth factor (VEGF), reviewed in [23]). PPARγ activity is governed by binding to small lipophilic ligands, such as polyunsaturated fatty acids and eicosanoids derived from the diet or metabolic pathways (e.g., the prostaglandin D2 metabolite 15-deoxy-12, 14-prostanglandin J2 (PGJ_2)) [27]. PPARγ is also activated by synthetic compounds called thiazolidinediones (TZDs), a class of insulin-sensitising agents. PPARγ may also be regulated by AMPK. Indeed, AMPK can phosphorylate PPARγ, repressing both the ligand-dependent and ligand-independent transactivating functions of this receptor [28].

PPARα is another isoform of PPAR expressed in the ovary. It regulates genes responsible for the uptake into cells and beta-oxidation of fatty acids [29]. Hypolipidaemic fibrate drugs, phthalate esters (plasticisers, herbicides), and long-chain polyunsaturated fatty acids and their lipooxygenase-derived metabolites (e.g., leukotriene) have been described as agonists of PPARα [30–32]. In vivo, fibrates are currently administrated alone or in combination with statins to patients with increased cardiovascular risk to impede the progression of atherosclerotic lesions. Insulin increases the transcriptional activity of PPARα by activating the MAPK pathway [33]. New therapeutics agents, such as glitazar, may activate both PPARα and PPARγ [34].

2.2. Structure and mechanisms of action of AMPK

Unlike PPARs, AMPK is a kinase comprised of three subunits: a catalytic subunit alpha and two regulatory subunits, beta and gamma [35]. The alpha subunit contains the catalytic core and binds, via its C-terminal tail, to the beta subunit, which serves as a docking subunit for the alpha and gamma subunits. AMPK is activated by a change in the AMP : ATP ratio within the cell and therefore acts as an efficient sensor of cellular energy state. This change in AMP : ATP ratio may result from exercise [36], hypoxia [37], hormones [38, 39], or the effects of pharmacological drugs, such as 5-aminoimidazole-4-carboxamide-riboside-5-phosphate (AICAR) [40]. Binding to AMP activates AMPK allosterically and induces phosphorylation of the threonine 172 residue of the α subunit by upstream kinases, including the tumour suppressor LKB1 [41, 42].

AMPK phosphorylates target proteins (including PPARγ) involved in a number of metabolic pathways, including lipid and cholesterol metabolism (adipocytes, liver, and muscle), glucose transport, glycogen, and protein metabolism (see review [35, 41]).

2.3. Involvement of PPARs and AMPK in the adiponectin action

AMPK and PPARα are both activated by adiponectin [11, 43] (Figure 1). Adiponectin (also known as apM1, AdipoQ,

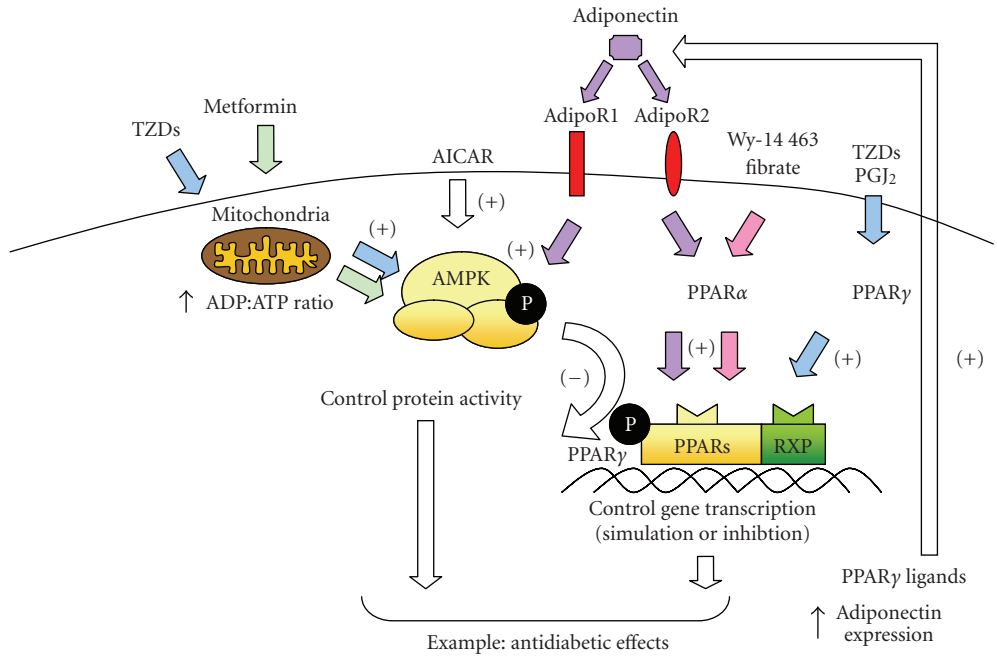


Figure 1: Schema illustrating the putative functional interactions between PPARs, AMPK, and adiponectin. PPARγ is activated by binding with PGJ_2or TZDs and PPARα with fibrates or WY 14 463. They control gene transcription, and, in particular, PPARγ ligands increase adiponectin expression [49]. Metformin and TZDs activate AMPK probably via the respiratory chain in mitochondria [22], and AICAR stimulates AMPK. AMPK controls protein activity by phosphorylation (e.g., inhibits PPARγ by phosphorylation [35]). Adiponectin activates AdipoR1 and AdipoR2 receptors which act on metabolism via AMPK (AdipoR1) or PPARα (AdipoR2) [43].

Gbp28, and Acrp30) is an adipocyte-derived factor [44, 45]. It is present as a multimer at high concentrations in the circulation (5 to 25 μg/ml in human [46]). In obese and type 2 diabetic humans, plasma adiponectin is strongly reduced suggesting that circulating adiponectin may be related to the development of insulin resistance [11]. Two adiponectin receptors (AdipoR1 and AdipoR2) have been identified in different tissues of various species. They each contain seven transmembrane domains, but are structurally and functionally different from G protein-coupled receptors. Adiponectin plays an important role in insulin sensitisation in mammals (inhibition of gluconeogenesis and stimulation of fatty acid oxidation) by activating AMPK [47] and PPARα proteins in skeletal muscle, liver, and adipocytes [43]. Thus, both TZDs and adiponectin have been shown to activate AMPK. Moreover, the promoter of the adiponectin gene contains a PPRE [48] and TZDs increase the production and plasma concentration of adiponectin [49]. TZDs have weaker antidiabetic effects in ob/ob mice lacking adiponectin gene than in ob/ob mice with adiponectin, and the activation of AMPK by TZDs is also attenuated in these mice, suggesting that adiponectin is required for the activation of AMPK by TZDs [50].

In porcine granulosa cells, adiponectin treatment induces the expression of genes associated with periovulatory remodeling of the ovarian follicle (cyclooxygenase-2, prostaglandin E synthase, and vascular endothelial growth factor [51]). Some of these genes are also activated by PPARγ. Furthermore, adiponectin receptors, PPARs, and AMPK are expressed in reproductive tissues, including the ovary.

3. EXPRESSION OF PPARs AND AMPK IN THE OVARY

3.1. Expression of PPARs in the ovary

All the PPAR isoforms are expressed in the ovary. The PPARα and PPARβ/δ isoforms are expressed primarily in the theca and stroma tissues [52], reviewed by [23], (see Table 1). The deletion of PPARα has no apparent effect on the fertility of mice, whereas PPARβ/δ-null mice present placental malformations leading to embryo death during early pregnancy [53–55]. PPARγ is expressed strongly in granulosa cells, and less strongly in the theca cells and corpus luteum, in the ovaries of rodents and ruminants (see Table 1) [52, 56, 57]. PPARγ is detected early in folliculogenesis (at the primary/secondary follicle stage) [58], and its expression increases until the large follicle stage and then decreases after the LH surge [58]. In mice, the absence of PPARγ in the ovaries results in lower levels of fertility [59]. No effect on folliculogenesis or ovulation rate has been observed, but fewer embryos implant, probably due to lower levels of progesterone production by the corpus luteum [59].

3.2. Expression of AMPK and adiponectin in the ovary

AMPK expression has been studied in the ovaries of various species, including rat [60, 65], mouse [61], cow [62], pig [63], and chicken [64]. RT-PCR has shown the mRNAs of all the AMPK subunits to be present in granulosa cells, the corpus luteum, oocyte, and cumulus-oocyte-complexes

Table 1: Location of PPARs, AMPK, and adiponectin in ovary.

	Species	Location	mRNA or Protein	References
PPARα	Rat	Theca and stroma		[52]
PPARβ/δ	Rat	Throughout the ovary		[52]
PPARγ	Mouse, rat, pig, sheep, cow, and human	Granulosa, corpus, luteum, porcine theca and granulosa cells oocytes		Reviewed by [23]
AMPK	Rat, cow, chicken, pig, mouse	Granulosa cells, oocyte, corpus luteum (weaker in rat theca cells for AMPK α1)	mRNA and protein	[60–64]
Adiponectin	Rat, chicken, pig	Theca cells, oocyte, and corpus luteum, Follicular liquid	mRNA (chicken) mRNA and protein (rat)	[12, 13, 51]
Adiponectin receptor I	Rat, chicken, pig	Granulosa and theca cells, oocyte and corpus luteum (rat)	mRNA (chicken) mRNA and protein (rat)	[12, 13, 51]
Adiponectin receptor II	Rat, chicken, pig	Granulosa cells, oocyte and corpus luteum (rat)	mRNA (chicken) mRNA and protein (rat)	[12, 13, 51]

in rodent and bovine ovaries (Table 1) [60, 62]. We have shown, by immunohistochemical analyses, that the AMPK α-subunit, like PPARγ, is more strongly expressed in granulosa cells than in theca cells in rats and cows [60, 62]. In cows, levels of AMPKα- and β-subunits were similar in small and large follicles. In hens, the activation of AMPK by its phosphorylation on the Thr172 residue increased during follicle development [64]. In mice, the absence of the catalytic AMPK alpha 2 subunit does not affect female fertility [66]. Until now, no data are available on the reproductive functions of the transgenic or knockout mice for the other subunits of AMPK.

In chicken ovary, adiponectin mRNA is more abundant in theca cells than in granulosa cells (Table 1) [13]. In porcine ovary, adiponectin is detected at similar concentrations in the follicular fluid and serum [51]. Both receptors are expressed in ovarian follicles. In chicken, the adiponectin type I receptor (AdipoRI) is twice as abundant in granulosa cells as in theca cells, and the type II receptor (AdipoR2) is expressed equally strongly in granulosa and thecal cells (Table 1) [13]. Studies in mice have shown that AdipoR1 may be more tightly linked to AMPK pathway activation, whereas AdipoR2 seems to be associated with PPARα activation [43]. However, mice lacking adiponectin [67], AdipoR1, AdipoR2, or both receptors [43] are fertile, which suggests that this signalling is not absolutely essential for ovarian function. However, it may be required for ovulation in other species or may simply be an additional component for fine-tuning ovarian function.

4. FUNCTION OF PPARs, AMPK, AND ADIPONECTIN IN THE OVARY

4.1. Regulation of steroidogenesis by PPARγ, PPARα, AMPK, and adiponectin

TZDs modulate cell proliferation and steroidogenesis in granulosa cells in vitro (reviewed by [23]). Sex steroid secretion (progesterone, oestradiol) may be inhibited by TZDs in sows and in women undergoing in vitro fertilization [56, 68] or stimulated (progesterone and oestradiol), as in rats and ewes [52, 57]). The effects of TZDs depend on the species and the status of granulosa cell differentiation (follicular phase, before or after the gonadotropin surge in human granulosa cells). TZDs could regulate their target genes at the transcriptional level (reviewed by [23, 68]). However, several studies have suggested that TZDs could also exert their effects by modifying the activity of steroidogenic enzymes (3-beta-hydroxysteroid-dehydrogenase (3-βHSD) and aromatase) [56, 69]. Indeed, the concentrations of Cyp11a1 and 3-βHSD mRNA in porcine granulosa cells and the levels of the corresponding proteins in ovine granulosa cells are not affected by TZD treatment [56, 57]. Moreover, TZDs increase the release of pregnenolone, a substrate of 3β-HSD, from porcine granulosa cells into the medium, whereas progesterone production decreases [56]. Ligands for PPARα are also known to alter ovarian steroidogenesis. For example, in vivo. fenofibrate, through PPARα-dependent mechanism, inhibits aromatase cytochrome P450 expression and activity in the ovary of mouse [70]. Another PPARα synthetic ligand, Wy-14 463, suppresses also aromatase transcript levels and oestradiol production in cultured rat granulosa cells [71].

AMPK, like PPARγ and PPARα, may influence ovarian function by modifying the synthesis of progesterone and oestradiol. Studies based on AICAR and the adenovirus-mediated expression of dominant negative AMPK have demonstrated that AMPK reduces progesterone production, but not oestradiol production, in rat granulosa cells [60]. This decrease is associated with a decrease in 3β-HSD mRNA and protein levels and a decrease in MAPK ERK1/2 phosphorylation [60]. Furthermore, the activation of AMPK by metformin decreases basal and FSH-induced progesterone secretion by decreasing the levels of proteins involved in steroidogenesis: (3βHSD, CYP11a1, STAR) [65]. In granulosa cells from humans and cows, metformin strongly decreases the secretion of progesterone and oestradiol [62, 72]. In bovine granulosa cells, this effect is mediated by AMPK

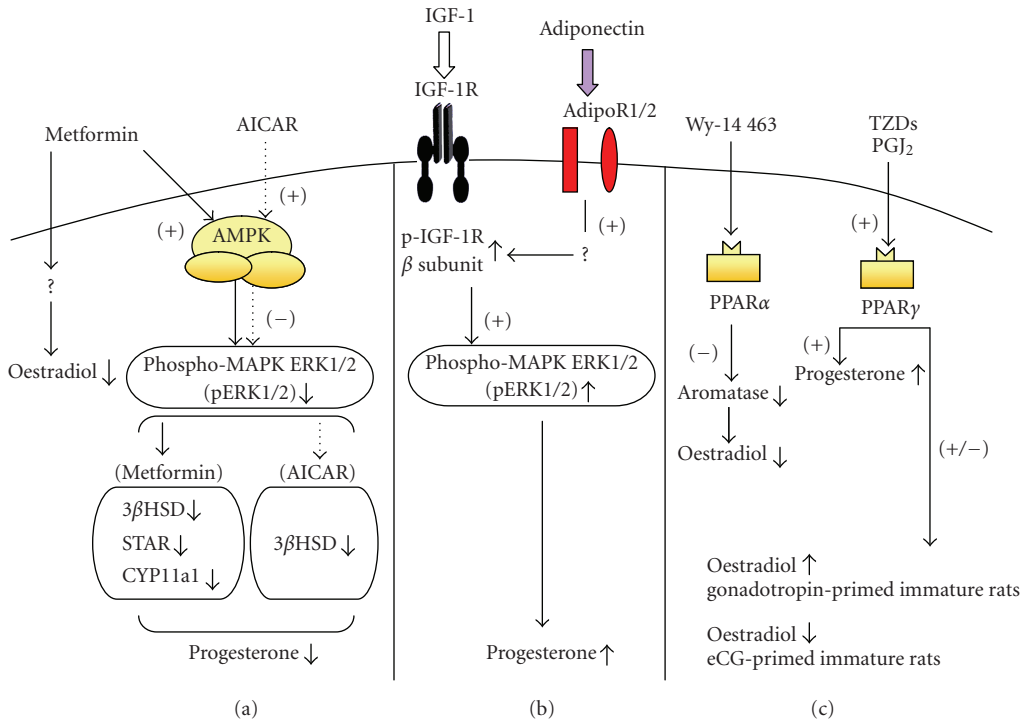


Figure 2: Schema illustrating the effects of (a) metformin- or AICAR-induced AMPK activation, (b)adiponectin, and (c) TZDs or PPAR alpha ligands on the rat granulosa cell steroidogenesis. (a) Metformin or AICAR treatment decreases MAPK ERK1/2 phosphorylation and progesterone secretion through AMPK activation [60, 65]. Metformin decreases also oestradiol secretion through an independent AMPK pathway [60]. (b) Adiponectin treatment increases IGF-1-induced IGF-1R β-subunit tyrosine phosphorylation and MAPK ERK1/2 phosphorylation and progesterone secretion [12]. (c) The PPARα ligand, Wy-14 463, inhibits oestradiol secretion whereas TZDs or PGJ2 increases progesterone secretion and inhibits estradiol secretion in eCG-primed immature rats or increases estradiol secretion in gonadotropin-primed immature rat [23, 52]. 3βHSD: 3β-hydroxysteroiddehydrogenase, STAR: Steroidogenic acute regulatory protein, CYP11a1: P450 sidechain cleavage, Adipo R1/2: Adiponectin receptor type I and II, MAPK ERK1/2: Mitogen Activated protein kinase Extracellular Regulated kinase,1/2, PGJ2: prostaglandine J2.

activation, and leads to a decrease in MAPK activation. In human granulosa cells, metformin also decreases androgen synthesis, by directly inhibiting Cyp17 activity [73].Thus, AMPK activation decreases steroidogenesis in the granulosa cells of various species. The effects of AMPK on steroid secretion, like those of PPARγ, depend on the species and the stimulator of AMPK (AICAR versus metformin). Several results suggest that metformin-induced AMPK activation could act through transcriptional mechanism. Further investigations are needed to determine the molecular mechanism of metformin.

Women treated for in vitro fertilization (IVF) present an increase in serum adiponectin concentration after the administration of human chorionic gonadotropin, this increase being correlated with progesterone levels [74]. In cultured porcine granulosa cells, adiponectin modulates the expression of genes encoding proteins involved in steroid production, increasing the abundance of transcripts for the steroidogenic acute regulatory protein, and decreasing the abundance of cytochrome P450 aromatase transcripts [51]. The MAPK pathway, rather than protein kinase A or AMPK, mediates the adiponectin signal in ovarian granulosa cells, by ERK1/2 phosphorylation [51]. Surprisingly, adiponectin alone does not affect steroid production in rat granulosa cells [12]. However, it approximately doubled the IGF-1-induced secretion of progesterone. These effects may be due to an increase in IGF-1 receptor beta subunit tyrosine phosphorylation and ERK1/2 phosphorylation [12]. A schema illustrating the effects of PPARα and γ, AMPK and adiponectin activation on the steroidogenesis of rat granulosa cells is shown in Figure 2.

4.2. Regulation of granulosa cell proliferation

In addition to their effects on steroidogenesis, TZDs decrease the proliferation of granulosa cells in sheep (PPARγ, [57]). These data are in good agreement with those obtained in bovine lutein cells since an aurintricarboxylic acid-mediated decrease of PPARγ is accompanied by a progression of the cell cycle [75]. In our knowledge, there are no data on the effects of PPARα ligands on granulosa cell proliferation. In contrast, AMPK and adiponectin are not essential for granulosa cell proliferation in rat [12, 60].

4.3. Regulation of oocyte maturation

PPARγ, AMPK, and adiponectin are all expressed in mammalian oocytes [12, 23, 60, 76]. However, AMPK has been

studied in more detail than PPARγ, PPARα, and adiponectin. PPARγ may regulate the expression of genes involved in the meiotic maturation of oocytes (e.g., nitric oxide synthase (NOS)) [23]. Wood et al. recently identified putative binding sites for PPARγ/RXR in the proximal promoters of several genes differentially expressed in oocytes from women with PCOS and known to play a role in the meiotic cell cycle [77]. All these results suggest that PPARγ/RXR may be active in the oocyte. The two adiponectin receptors, AdipoR1 and AdipoR2, are also expressed in rat oocytes, and AMPK activity has also been detected in oocytes of several species (see above), suggesting that adiponectin may play a role through AMPK in determining oocyte quality (cited by [78]). In addition, women with PCOS showing impairment in the final maturation of oocytes and in ovulation, present lower circulating concentrations of adiponectin [10, 79].

In vivo, the oocyte remains at the immature stage or germinal vesicle stage (GV, i.e., prophase of meiosis I) until the preovulatory LH surge [79]. However, if cumulus-oocyte complexes (COCs) are removed from the follicles and cultured in vitro, oocytes may spontaneously resume meiosis [80, 81]. During nuclear maturation, immature oocytes undergo germinal vesicle breakdown (GVBD) and proceed through metaphase II of meiosis. The pharmacological activation of AMPK, by AICAR injection, in mouse oocytes leads to the induction of oocyte maturation in arrested cumulus-enclosed oocytes [82]. Metabolic stresses (oxidative or osmotic) known to activate AMPK accelerate meiosis in oocytes in which meiosis was previously arrested by cAMP analogues [83]. However, the data for mice conflict with those obtained with porcine and bovine oocytes [84, 85]. Indeed, in these two latter species, AICAR and metformin significantly increase phosphorylation/activation of AMPK and the percentage of COCs arrested at the GV stage. Thus, AMPK activation has opposite effects in the control of oocyte maturation in cows, sows and mice. This could be explained by the important differences that exist in the regulation of oocyte meiotic resumption between rodent and nonrodent animals such as for example the time taken for oocytes to undergo meiotic resumption (2 to 3 hours of in vitro maturation in rodent, 20 hours in pig, and 22 hours in bovine species). Interestingly, in women with PCOS treated with metformin, the number of mature oocytes retrieved and oocytes fertilized has been shown to increase after gonadotropin stimulation for IVF [86]. However, recent data indicate that clomiphene is superior to metformin in achieving live birth in infertile women with PCOS [87].

5. CONCLUSION

The nuclear PPARs and the fuel sensor AMPK are expressed in the ovary of various species. Several studies have shown that they modulate ovarian cell proliferation and steroidogenesis and could be involved in oocyte maturation. Both PPARα and AMPK mediate the effects of hormones involved in lipid and glucose metabolism, including adiponectin. Thus, PPARs, AMPK, and adiponectin may be key signals regulating the amount of energy required for the growth of follicles, oocytes, and embryos. Further investigations are necessary to assess the exact importance and mechanisms of action of these molecules in some ovarian dysfunctions including for example PCOS syndrome.

ACKNOWLEDGMENTS

This work was partly supported by the GIS-AGENA, ANR, and Apis-GENE.

REFERENCES

[1] W. R. Butler, "Nutritional interactions with reproductive performance in dairy cattle," *Animal Reproduction Science*, vol. 60-61, pp. 449–457, 2000.

[2] R. Fernandez-Fernandez, A. C. Martini, V. M. Navarro, et al., "Novel signals for the integration of energy balance and reproduction," *Molecular and Cellular Endocrinology*, vol. 254-255, pp. 127–132, 2006.

[3] D. J. Burks, J. F. de Mora, M. Schubert, et al., "IRS-2 pathways integrate female reproduction and energy homeostasis," *Nature*, vol. 407, no. 6802, pp. 377–382, 2000.

[4] J. C. Bruning, D. Gautam, D. J. Burks, et al., "Role of brain insulin receptor in control of body weight and reproduction," *Science*, vol. 289, no. 5487, pp. 2122–2125, 2000.

[5] A. Dunaif, "Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis," *Endocrine Reviews*, vol. 18, no. 6, pp. 774–800, 1997.

[6] M. J. Iuorno and J. E. Nestler, "Insulin-lowering drugs in polycystic ovary syndrome," *Obstetrics and Gynecology Clinics of North America*, vol. 28, no. 1, pp. 153–164, 2001.

[7] E. Seli and A. J. Duleba, "Treatment of PCOS with metformin and other insulin-sensitizing agents," *Current Diabetes Reports*, vol. 4, no. 1, pp. 69–75, 2004.

[8] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[9] N. Musi, M. F. Hirshman, J. Nygren, et al., "Metformin increases AMP-activated protein-kinase activity in skeletal muscle of subjects with type 2 diabetes," *Diabetes*, vol. 51, no. 7, pp. 2074–2081, 2002.

[10] M. S. Ardawi and A. A. Rouzi, "Plasma adiponectin and insulin resistance in women with polycystic ovary syndrome," *Fertility and Sterility*, vol. 83, no. 6, pp. 1708–1716, 2005.

[11] T. Kadowaki and T. Yamauchi, "Adiponectin and adiponectin receptors," *Endocrine Reviews*, vol. 26, no. 3, pp. 439–451, 2005.

[12] C. Chabrolle, L. Tosca, and J. Dupont, "Regulation of adiponectin and its receptors in rat ovary by human chorionic gonadotrophin treatment and potential involvement of adiponectin in granulosa cell steroidogenesis," *Reproduction*, vol. 133, no. 4, pp. 719–731, 2007.

[13] C. Chabrolle, L. Tosca, S. Crochet, S. Tesseraud, and J. Dupont, "Expression of adiponectin and its receptors (AdipoR1 and AdipoR2) in chicken ovary: potential role in ovarian steroidogenesis," to appear in *Domestic Animal Endocrinology*.

[14] R. Mattos, C. R. Staples, and W. W. Thatcher, "Effects of dietary fatty acids on reproduction in ruminants," *Reviews of Reproduction*, vol. 5, no. 1, pp. 38–45, 2000.

[15] C. M. Garcia-Bojalil, C. R. Staples, C. A. Risco, J. D. Savio, and W. W. Thatcher, "Protein degradability and calcium salts of long-chain fatty acids in the diets of lactating dairy cows:

reproductive responses," *Journal of Dairy Science*, vol. 81, no. 5, pp. 1385–1395, 1998.

[16] B. P. Kota, T. H.-W. Huang, and B. D. Roufogalis, "An overview on biological mechanisms of PPARs," *Pharmacological Research*, vol. 51, no. 2, pp. 85–94, 2005.

[17] D. G. Hardie and D. Carling, "The AMP-activated protein kinase—fuel gauge of the mammalian cell?" *European Journal of Biochemistry*, vol. 246, no. 2, pp. 259–273, 1997.

[18] S. Han and J. Roman, "Rosiglitazone suppresses human lung carcinoma cell growth through PPARγ-dependent and PPARγ-independent signal pathways," *Molecular Cancer Therapeutics*, vol. 5, no. 2, pp. 430–437, 2006.

[19] N. K. LeBrasseur, M. Kelly, T.-S. Tsao, et al., "Thiazolidinediones can rapidly activate AMP-activated protein kinase in mammalian tissues," *American Journal of Physiology*, vol. 291, no. 1, pp. E175–E181, 2006.

[20] L. G. Fryer, A. Parbu-Patel, and D. Carling, "The anti-diabetic drugs rosiglitazone and metformin stimulate AMP-activated protein kinase through distinct signaling pathways," *Journal of Biological Chemistry*, vol. 277, no. 28, pp. 25226–25232, 2002.

[21] G. Pilon, P. Dallaire, and A. Marette, "Inhibition of inducible nitric-oxide synthase by activators of AMP-activated protein kinase: a new mechanism of action of insulin-sensitizing drugs," *Journal of Biological Chemistry*, vol. 279, no. 20, pp. 20767–20774, 2004.

[22] M. R. Owen, E. Doran, and A. P. Halestrap, "Evidence that metformin exerts its anti-diabetic effects through inhibition of complex 1 of the mitochondrial respiratory chain," *Biochemical Journal*, vol. 348, no. 3, pp. 607–614, 2000.

[23] C. M. Komar, "Peroxisome proliferator-activated receptors (PPARs) and ovarian function—implications for regulating steroidogenesis, differentiation, and tissue remodeling," *Reproductive Biology and Endocrinology*, vol. 3, pp. 31, 2005.

[24] K. S. Miyata, S. E. McCaw, S. L. Marcus, R. A. Rachubinski, and J. P. Capone, "The peroxisome proliferator-activated receptor interacts with the retinoid X receptor in vivo," *Gene*, vol. 148, no. 2, pp. 327–330, 1994.

[25] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[26] F. Picard and J. Auwerx, "PPARγ and glucose homeostasis," *Annual Review of Nutrition*, vol. 22, pp. 167–197, 2002.

[27] Y. Kobayashi, S. Ueki, G. Mahemuti, et al., "Physiological levels of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 prime eotaxin-induced chemotaxis on human eosinophils through peroxisome proliferator-activated receptor-γ ligation," *Journal of Immunology*, vol. 175, no. 9, pp. 5744–5750, 2005.

[28] T. Leff, "AMP-activated protein kinase regulates gene expression by direct phosphorylation of nuclear proteins," *Biochemical Society Transactions*, vol. 31, part 1, pp. 224–227, 2003.

[29] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[30] G. Krey, O. Braissant, F. L'Horset, et al., "Fatty acids, eicosanoids, and hypolipidemic agents identified as ligands of peroxisome proliferator-activated receptors by coactivator-dependent receptor ligand assay," *Molecular Endocrinology*, vol. 11, no. 6, pp. 779–791, 1997.

[31] K. Schoonjans, B. Staels, and J. Auwerx, "Role of the peroxisome proliferator-activated receptor (PPAR) in mediating the effects of fibrates and fatty acids on gene expression," *Journal of Lipid Research*, vol. 37, no. 5, pp. 907–925, 1996.

[32] Y.-C. Zhou and D. J. Waxman, "Activation of peroxisome proliferator-activated receptors by chlorinated hydrocarbons and endogenous steroids," *Environmental Health Perspectives*, vol. 106, supplement 4, pp. 983–988, 1998.

[33] A. Shalev, C. A. Siegrist-Kaiser, P. M. Yen, et al., "The peroxisome proliferator-activated receptor α is a phosphoprotein: regulation by insulin," *Endocrinology*, vol. 137, no. 10, pp. 4499–4502, 1996.

[34] C. Fiévet, J.-C. Fruchart, and B. Staels, "PPARα and PPARγ dual agonists for the treatment of type 2 diabetes and the metabolic syndrome," *Current Opinion in Pharmacology*, vol. 6, no. 6, pp. 606–614, 2006.

[35] D. G. Hardie, "The AMP-activated protein kinase pathway—new players upstream and downstream," *Journal of Cell Science*, vol. 117, no. 23, pp. 5479–5487, 2004.

[36] T. Hayashi, M. F. Hirshman, E. J. Kurth, W. W. Winder, and L. J. Goodyear, "Evidence for 5' AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport," *Diabetes*, vol. 47, no. 8, pp. 1369–1373, 1998.

[37] J. Mu, J. T. Brozinick Jr., O. Valladares, M. Bucan, and M. J. Birnbaum, "A role for AMP-activated protein kinase in contraction- and hypoxia-regulated glucose transport in skeletal muscle," *Molecular Cell*, vol. 7, no. 5, pp. 1085–1094, 2001.

[38] T. Yamauchi, J. Kamon, Y. Minokoshi, et al., "Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase," *Nature Medicine*, vol. 8, no. 11, pp. 1288–1295, 2002.

[39] Y. Minokoshi, Y.-B. Kim, O. D. Peroni, et al., "Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase," *Nature*, vol. 415, no. 6869, pp. 339–343, 2002.

[40] J. M. Corton, J. G. Gillespie, S. A. Hawley, and D. G. Hardie, "5-aminoimidazole-4-carboxamide ribonucleoside—a specific method for activating AMP-activated protein kinase in intact cells?" *European Journal of Biochemistry*, vol. 229, no. 2, pp. 558–565, 1995.

[41] B. B. Kahn, T. Alquier, D. Carling, and D. G. Hardie, "AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism," *Cell Metabolism*, vol. 1, no. 1, pp. 15–25, 2005.

[42] D. G. Hardie, "New roles for the LKB1→ AMPK pathway," *Current Opinion in Cell Biology*, vol. 17, no. 2, pp. 167–173, 2005.

[43] T. Yamauchi, Y. Nio, T. Maki, et al., "Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions," *Nature Medicine*, vol. 13, no. 3, pp. 332–339, 2007.

[44] P. E. Scherer, S. Williams, M. Fogliano, G. Baldini, and H. F. Lodish, "A novel serum protein similar to C1q, produced exclusively in adipocytes," *Journal of Biological Chemistry*, vol. 270, no. 45, pp. 26746–26749, 1995.

[45] K. Maeda, K. Okubo, I. Shimomura, T. Funahashi, Y. Matsuzawa, and K. Matsubara, "cDNA cloning and expression of a novel adipose specific collagen-like factor, apM1," *Biochemical and Biophysical Research Communications*, vol. 221, no. 2, pp. 286–289, 1996.

[46] U. B. Pajvani and P. E. Scherer, "Adiponectin: systemic contributor to insulin sensitivity," *Current Diabetes Reports*, vol. 3, no. 3, pp. 207–213, 2003.

[47] T. Yamauchi, J. Kamon, Y. Minokoshi, et al., "Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase," *Nature Medicine*, vol. 8, no. 11, pp. 1288–1295, 2002.

[48] M. Iwaki, M. Matsuda, N. Maeda, et al., "Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors," *Diabetes*, vol. 52, no. 7, pp. 1655–1663, 2003.

[49] N. Maeda, M. Takahashi, T. Funahashi, et al., "PPARγ ligands increase expression and plasma concentrations of adiponectin, an adipose-derived protein," *Diabetes*, vol. 50, no. 9, pp. 2094–2099, 2001.

[50] A. R. Nawrocki, M. W. Rajala, E. Tomas, et al., "Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor γ agonists," *Journal of Biological Chemistry*, vol. 281, no. 5, pp. 2654–2660, 2006.

[51] S. Ledoux, D. B. Campos, F. L. Lopes, M. Dobias-Goff, M.-F. Palin, and B. D. Murphy, "Adiponectin induces periovulatory changes in ovarian follicular cells," *Endocrinology*, vol. 147, no. 11, pp. 5178–5186, 2006.

[52] C. M. Komar, O. Braissant, W. Wahli, and T. E. Curry Jr., "Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period," *Endocrinology*, vol. 142, no. 11, pp. 4831–4838, 2001.

[53] S. S. Lee, T. Pineau, J. Drago, et al., "Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators," *Molecular and Cellular Biology*, vol. 15, no. 6, pp. 3012–3022, 1995.

[54] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[55] J. M. Peters, S. S. Lee, W. Li, et al., "Growths, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor β(δ)," *Molecular and Cellular Biology*, vol. 20, no. 14, pp. 5119–5128, 2000.

[56] S. Gasic, Y. Bodenburg, M. Nagamani, A. Green, and R. J. Urban, "Troglitazone inhibits progesterone production in porcine granulosa cells," *Endocrinology*, vol. 139, no. 12, pp. 4962–4966, 1998.

[57] P. Froment, S. Fabre, J. Dupont, et al., "Expression and functional role of peroxisome proliferator-activated receptor-γ in ovarian folliculogenesis in the sheep," *Biology of Reproduction*, vol. 69, no. 5, pp. 1665–1674, 2003.

[58] C. Komar, "Initiation of peroxysome proliferator-activated receptor gamma (PPARg) expression in the neonatal rat ovary," in *Proceedings of the 38th Annual Meeting of the Society for the Study of Reproduction*, Quebec City, Quebec, Canada, July 2005.

[59] Y. Cui, K. Miyoshi, E. Claudio, et al., "Loss of the peroxisome proliferation-activated receptor gamma (PPARγ) does not affect mammary development and propensity for tumor formation but leads to reduced fertility," *Journal of Biological Chemistry*, vol. 277, no. 20, pp. 17830–17835, 2002.

[60] L. Tosca, P. Froment, P. Solnais, P. Ferré, F. Foufelle, and J. Dupont, "Adenosine 5′-monophosphate-activated protein kinase regulates progesterone secretion in rat granulosa cells," *Endocrinology*, vol. 146, no. 10, pp. 4500–4513, 2005.

[61] S. M. Downs, E. R. Hudson, and D. G. Hardie, "A potential role for AMP-activated protein kinase in meiotic induction in mouse oocytes," *Developmental Biology*, vol. 245, no. 1, pp. 200–212, 2002.

[62] L. Tosca, C. Chabrolle, S. Uzbekova, and J. Dupont, "Effects of metformin on bovine granulosa cells steroidogenesis: possible involvement of adenosine 5′ monophosphate-activated protein kinase (AMPK)," *Biology of Reproduction*, vol. 76, no. 3, pp. 368–378, 2007.

[63] M. A. Mayes, M. F. Laforest, C. Guillemette, R. B. Gilchrist, and F. J. Richard, "Adenosine 5′-monophosphate kinase-activated protein kinase (PRKA) activators delay meiotic resumption in porcine oocytes," *Biology of Reproduction*, vol. 76, no. 4, pp. 589–597, 2007.

[64] L. Tosca, S. Crochet, P. Ferré, F. Foufelle, S. Tesseraud, and J. Dupont, "AMP-activated protein kinase activation modulates progesterone secretion in granulosa cells from hen preovulatory follicles," *Journal of Endocrinology*, vol. 190, no. 1, pp. 85–97, 2006.

[65] L. Tosca, P. Solnais, P. Ferré, F. Foufelle, and J. Dupont, "Metformin-induced stimulation of adenosine 5′ monophosphate-activated protein kinase (PRKA) impairs progesterone secretion in rat granulosa cells," *Biology of Reproduction*, vol. 75, no. 3, pp. 342–351, 2006.

[66] B. Viollet, F. Andreelli, S. B. Jørgensen, et al., "The AMP-activated protein kinase α2 catalytic subunit controls whole-body insulin sensitivity," *Journal of Clinical Investigation*, vol. 111, no. 1, pp. 91–98, 2003.

[67] K. Ma, A. Cabrero, P. K. Saha, et al., "Increased β-oxidation but no insulin resistance or glucose intolerance in mice lacking adiponectin," *Journal of Biological Chemistry*, vol. 277, no. 38, pp. 34658–34661, 2002.

[68] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[69] Y.-M. Mu, T. Yanase, Y. Nishi, et al., "Insulin sensitizer, troglitazone, directly inhibits aromatase activity in human ovarian granulosa cells," *Biochemical and Biophysical Research Communications*, vol. 271, no. 3, pp. 710–713, 2000.

[70] K. Toda, T. Okada, C. Miyaura, and T. Saibara, "Fenofibrate, a ligand for PPARα , inhibits aromatase cytochrome P450 expression in the ovary of mouse," *Journal of Lipid Research*, vol. 44, no. 2, pp. 265–270, 2003.

[71] T. N. Lovekamp and B. J. Davis, "Mono-(2-ethylhexyl) phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells," *Toxicology and Applied Pharmacology*, vol. 172, no. 3, pp. 217–224, 2001.

[72] R. Mansfield, R. Galea, M. Brincat, D. Hole, and H. Mason, "Metformin has direct effects on human ovarian steroidogenesis," *Fertility and Sterility*, vol. 79, no. 4, pp. 956–962, 2003.

[73] A. La Marca, T. O. Egbe, G. Morgante, T. Paglia, A. Ciani, and V. De Leo, "Metformin treatment reduces ovarian cytochrome P-450c17α response to human chorionic gonadotrophin in women with insulin resistance-related polycystic ovary syndrome," *Human Reproduction*, vol. 15, no. 1, pp. 21–23, 2000.

[74] Y.-H. Liu, E.-M. Tsai, Y.-L. Chen, et al., "Serum adiponectin levels increase after human chorionic gonadotropin treatment during in vitro fertilization," *Gynecologic and Obstetric Investigation*, vol. 62, no. 2, pp. 61–65, 2006.

[75] B. Löhrke, T. Viergutz, S. K. Shahi, et al., "Detection and functional characterisation of the transcription factor peroxisome proliferator-activated receptor γ in lutein cells," *Journal of Endocrinology*, vol. 159, no. 3, pp. 429–439, 1998.

[76] M. Mohan, S. Ryder, P. L. Claypool, R. D. Geisert, and J. R. Malayer, "Analysis of gene expression in the bovine blastocyst

produced in vitro using suppression-subtractive hybridization," *Biology of Reproduction*, vol. 67, no. 2, pp. 447–453, 2002.

[77] J. R. Wood, D. A. Dumesic, D. H. Abbott, and J. F. Strauss III, "Molecular abnormalities in oocytes from women with polycystic ovary syndrome revealed by microarray analysis," *Journal of Clinical Endocrinology and Metabolism*, vol. 92, no. 2, pp. 705–713, 2007.

[78] M. Mitchell, D. T. Armstrong, R. L. Robker, and R. J. Norman, "Adipokines: implications for female fertility and obesity," *Reproduction*, vol. 130, no. 5, pp. 583–597, 2005.

[79] T. Sir-Petermann, B. Echiburú, M. M. Maliqueo, et al., "Serum adiponectin and lipid concentrations in pregnant women with polycystic ovary syndrome," *Human Reproduction*, vol. 22, no. 7, pp. 1830–1836, 2007.

[80] G. Pincus and E. V. Enzmann, "The comparative behavior of mammalian eggs in vivo and in vitro," *The Journal of Experimental Medicine*, vol. 62, pp. 665–675, 1935.

[81] R. G. Edwards, "Maturation in vitro of mouse, sheep, cow, pig, rhesus monkey and human ovarian oocytes," *Nature*, vol. 208, no. 8, pp. 349–351, 1965.

[82] J. Chen, E. Hudson, M. M. Chi, et al., "AMPK regulation of mouse oocyte meiotic resumption in vitro," *Developmental Biology*, vol. 291, no. 2, pp. 227–238, 2006.

[83] C. LaRosa and S. M. Downs, "Stress stimulates AMP-activated protein kinase and meiotic resumption in mouse oocytes," *Biology of Reproduction*, vol. 74, no. 3, pp. 585–592, 2006.

[84] S. Bilodeau-Goeseels, M. Sasseville, C. Guillemette, and F. J. Richard, "Effects of adenosine monophosphate-activated kinase activators on bovine oocyte nuclear maturation in vitro," *Molecular Reproduction and Development*, vol. 74, no. 8, pp. 1021–1034, 2007.

[85] L. Tosca, S. Uzbekova, C. Chabrolle, and J. Dupont, "Possible role of AMPK in the metformin-mediated arrest of bovine oocytes at the GV stage during in vitro maturation," to appear in *Biology of Reproduction*.

[86] L. A. Stadtmauer, S. K. Toma, R. M. Riehl, and L. M. Talbert, "Impact of metformin therapy on ovarian stimulation and outcome in 'coasted' patients with polycystic ovary syndrome undergoing in-vitro fertilization," *Reproductive Biomedicine Online*, vol. 5, no. 2, pp. 112–116, 2002.

[87] R. S. Legro, H. X. Barnhart, W. D. Schlaff, et al., "Clomiphene, metformin, or both for infertility in the polycystic ovary syndrome," *New England Journal of Medicine*, vol. 356, no. 6, pp. 551–566, 2007.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 142082, 11 pages
doi:10.1155/2008/142082

Review Article

PPAR Signaling in Placental Development and Function

Yaacov Barak,[1] Yoel Sadovsky,[2] and Tali Shalom-Barak[1]

[1] *The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA*
[2] *Departments of Obstetrics and Gynecology and Cell Biology and Physiology, Washington University School of Medicine, P.O. Box 8064, 4566 Scott Avenue, St. Louis, MO 63110, USA*

Correspondence should be addressed to Yaacov Barak, yaki.barak@jax.org

Received 4 June 2007; Accepted 28 August 2007

Recommended by P. Froment

With the major attention to the pivotal roles of PPARs in diverse aspects of energy metabolism, the essential functions of PPARγ and PPARβ/δ in placental development came as a surprise and were often considered a nuisance en route to their genetic analysis. However, these findings provided an opportune entrée into placental biology. Genetic and pharmacological studies, primarily of knockout animal models and cell culture, uncovered networks of PPARγ and PPARδ, their heterodimeric RXR partners, associated transcriptional coactivators, and target genes, that regulate various aspects of placental development and function. These studies furnish both specific information about trophoblasts and the placenta and potential hints about the functions of PPARs in other tissues and cell types. They reveal that the remarkable versatility of PPARs extends beyond the orchestration of metabolism to the regulation of cellular differentiation, tissue development, and trophoblast-specific functions. This information and its implications are the subject of this review.

1. INTRODUCTION

Mammalian reproduction entails prolonged gestation, posing the challenge of securing the thrift and long-term survival of the fetus in utero. The evolutionary answer to this challenge has been the emergence of the placenta, whose roles are to facilitate efficient nutrient, gas and waste exchange between the mother and fetus, while conferring immune privilege on the embryo and secreting pregnancy hormones. The placental core comprises a dense vascular array, where maternal and fetal circulations run in close proximity, but are strictly separated by a trophoblast barrier that specializes in essential bidirectional metabolite transport into and out of the fetus. Placental dysfunction is associated with common disorders of pregnancy, including spontaneous abortions, intrauterine growth restriction (IUGR), and preeclampsia, all of which are commonly associated with compromised placental vasculature [1–3]. In the mouse, dozens of targeted gene mutations result in placental defects that underlie stunted growth or midgestation lethality (reviewed in [4, 5]). Proof of direct causative relationship between such defects and the lethal outcome comes from the complete rescue of embryos by selective reconstitution of the trophoblast in several knockout mouse strains [6–12].

Among the genes whose deficiency results in lethal placental defects are PPARγ and PPARδ; the two are closely related, yet functionally distinct members of the nuclear hormone receptor superfamily of ligand-activated transcription factors. Obligate heterodimers of PPARs and retinoid X receptors (RXRs) bind to PPAR-response elements (PPREs) in the cis-regulatory regions of target genes and activate transcription in response to small lipophilic ligands. While the identities of endogenous PPAR ligands are still inconclusive, pharmaceutical development has yielded several high-affinity synthetic agonists that are widely used in both the clinic and the lab. Importantly, notwithstanding the primary focus of the PPAR field on cellular and systemic metabolism, PPARs and their associated regulators play at least equally essential roles in placental development and function, as reviewed below.

1.1. Placental development and trophoblast differentiation

The deepest insights into the functions of PPARs in the placenta have been provided by mouse genetic studies. This succinct overview and the accompanying Figure 1 aim at providing the framework for these studies by summarizing

placental development in mice. One should bear in mind that while basic principles and molecular regulation of placental development and function are similar across mammals, morphological patterning and architecture of the placenta, and hence terminology, vary considerably among species.

With the exception of the percolating maternal blood, the placenta is exclusively an embryonic tissue. The juxtaposed decidua is a maternal tissue formed from endometrial lining of the uterus. The placenta is comprised of trophoblast cells that originate from the trophectoderm layer of the blastocyst (Figure 1). Implantation of the embryo into the uterine wall triggers the expansion and initial differentiation of trophectoderm cells to form both the chorion and, by process of endoreduplication, primary giant cells. These giant cells facilitate uterine invasion by the embryo. The chorion harbors trophoblast stem cells and, in the mouse, gives rise to the ectoplacental cone (EPC). After initial expansion, the EPC yields the spongiotrophoblast layer and secondary giant cells (Figure 1). Giant cells separate the placenta from the maternal decidua and are responsible both for maintaining the tight placenta-decidua interface and for executing various endocrine functions, including secretion of steroid and prolactin family pregnancy hormones. Spongiotrophoblasts perform (a) endocrine functions by secreting pregnancy specific glycoproteins (PSGs) and prolactin-related hormones, (b) metabolic functions, such as glycogen storage and production of IGF2, and (c) presumed mechanical support functions. Syncytiotrophoblasts that comprise the hemochorial trophoblast barrier between maternal and embryonic circulations (the labyrinthine layer in mice; floating chorionic villi in humans) originate directly from the chorion. In the mouse, vascularization of the placenta initiates around E8.5, when the allantois, which harbors the future umbilical blood vessels, attaches to the chorionic plate. Subsequently, the chorioallantois invaginates into the placenta and lays the vascular framework of the labyrinth. Concomitantly, chorionic trophoblasts in the labyrinth differentiate into three morphologically and functionally distinct single cell layers that form a highly specialized epithelial barrier, which execute all bidirectional transport functions between the mother and the fetus. Insights from mouse mutants demonstrate that formations of the labyrinthine trophoblast and placental vascularization are highly concordant and involve extensive cellular and molecular interactions between the allantoic endothelium and the trophoblast [4]. The trophoblast is crucial for placental vascularization, as evident from the complete correction of diverse placental vascular defects by trophoblast-selective rescue [8–12]. In turn, multiple signaling factors secreted by the embryonic endothelium, such as HGF, EGF, LIF, PDGFB, and WNT-2, are essential for proper formation of the labyrinth [13–20].

Cell culture studies have facilitated the mechanistic understanding of molecular and cellular processes involved in various aspects of trophoblast differentiation and function. This area has been markedly advanced by the successful establishment of protocols for procuring and manipulating trophoblast stem (TS) cells from blastocysts or the EPC [21]. The stem cell status of TS cells can be maintained by FGF4 and embryonic fibroblast-derived factors, possibly related to TGFβ or activin [21, 22]. When FGF and conditioned media are withdrawn from the culture medium, mimicking the growing distance between distal trophoblast layers and the embryonic FGF4 source, TS cells differentiate spontaneously, primarily into giant cells and to some extent also into spongiotrophoblast and multinucleated syncytial cells [21, 23]. Moreover, when reintroduced into blastocysts, TS cells are able to differentiate into all trophoblast derivatives [21], demonstrating their true stem cell nature.

2. PPARγ

In the absence of prior evidence that PPARγ is expressed during early embryogenesis, the death of *Pparg*-null embryos at the 10th day of gestation (E10.0) was initially surprising [12]. However, further inquiry revealed that *Pparg* is expressed abundantly in the placenta from E8.5 onward, and is not detected in any other embryonic tissue until at least E13.5 (12). This expression pattern provided circumstantial evidence that PPARγ may function in the placenta, but the survival of tetraploid chimeras provided the definitive proof that placental PPARγ deficiency was the cause of embryonic lethality [12]. Tetraploid chimeras are generated by electrofusing 2-cell embryos into single cells with tetraploid genomes. Such embryos resume development, and their aggregation with diploid morulas or embryonic stem cells gives rise to chimeras whose embryo derives exclusively from the diploid partner while their placentas derive from the tetraploid partners [24]. When used to reconstitute diploid *Pparg*-null embryos with WT tetraploid placentas, this procedure allowed survival of the mutant embryos until birth, when they succumbed to unrelated defects that included severe cerebral and intestinal hemorrhages [12]. The recent availability of epiblast-specific Cre transgenes, which delete loxP-flanked (floxed) alleles efficiently in the embryo but not extraembryonic tissue, has enabled to reprove this notion by demonstrating that near-complete deficiency of *Pparg* in the embryo proper is not embryonic lethal [25, 26].

2.1. PPARγ and trophoblast differentiation

The complex histological and ultrastructural phenotype of *Pparg*-null placentas (Figure 2) provided insights into the essential functions of PPARγ. Expression and spatial distribution of prototypic trophoblast lineage markers are intact in the mutant placentas, including the giant cell layer, the spongiotrophoblast, the labyrinth, and the chorion [12]. However, labyrinthine trophoblast precursors fail to terminally differentiate, and instead, retain parenchymal morphology without undergoing either compaction or syncytium formation [12]. The basement membrane between the trophoblast and fetal endothelium is severely disrupted, loosening the critical tight association between the two cell types [12]. This defect likely hampers both the flow of metabolites from the trophoblast to the embryo and the ability of embryonic vessels to use basement membrane tracks for extending and branching into the labyrinth. Consequently, fetal vessels do not permeate the *Pparg*-null placenta and the labyrinthine layer does not effectively form [12]. The trophoblast-lined

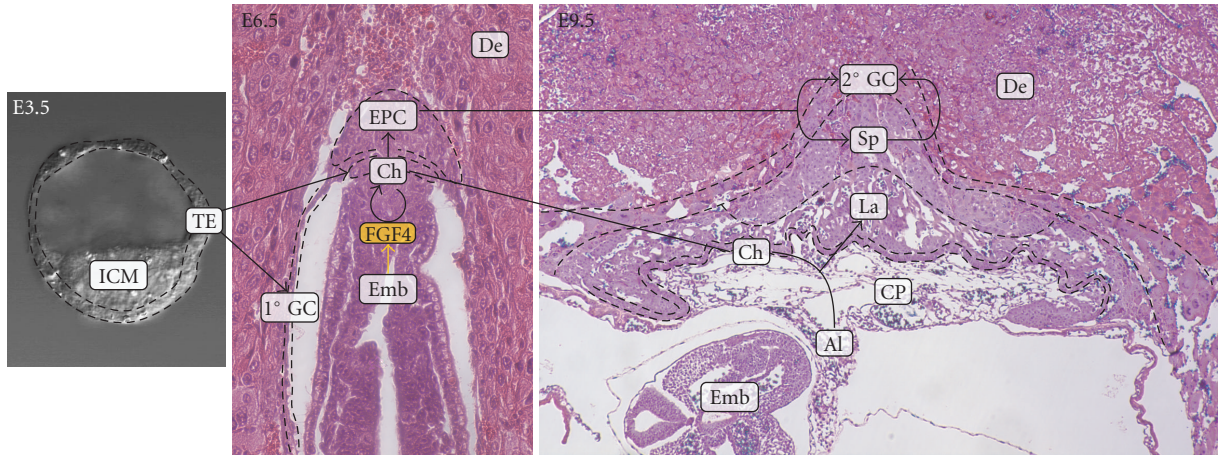


Figure 1: Trophoblast lineages in the developing mouse placenta. Shown from left to right are a blastocyst (E3.5), an E6.5 embryo, and an E9.5 embryo. Respective trophoblast lineages are traced for clarity. Al: allantois; Ch: chrion; CP: chorionic plate; De: decidua; Emb: embryo; EPC: ectoplacental cone; 1°GC: primary giant cells; 2°GC: secondary giant cells; ICM: inner cell mass; La: labyrinth; Sp: spongiotrophoblast; TE: trophectoderm. FGF4: fibroblast growth factor 4 secreted by the embryo to maintain the chorion. Blastocyst and E6.5 embryo picture courtesy of Drs. Mimi DeVries and Tom Gridley, respectively, The Jackson Laboratory.

maternal blood pools are dilated and ruptured, leading to hemorrhages, fibrin deposition, and overt phagocytosis of maternal erythrocytes by junctional zone trophoblasts [12]. Together, these observations indicate that while PPARγ is dispensable for partition of trophoblasts to different lineages, it is essential for terminal differentiation of labyrinthine syncytiotrophoblasts and spongiotrophoblasts, and in turn for placental vascularization and integrity. The further increase of *Pparg* levels in the labyrinth during late gestation suggests that beyond its role in establishing the vascular network of the placenta it may also play an important role in its elaboration and maintenance [27].

On the opposite pole of the PPARγ spectrum, feeding pregnant mice a high dose of the PPARγ agonist rosiglitazone (rosi) from mid to late gestation elicited severe thinning of the spongiotrophoblast layer and substantial dilation of the maternal blood pools in WT placentas [28]. *Pparg*$^{+/-}$ placentas were protected from these effects, indicating that these are indeed the result of excessive PPARγ activity. Reduced expression of the trophoblast stem cell marker *Eomes* in rosi-treated WT placentas [28] suggested that excessive PPARγ activity might cause these effects by accelerating stem cell differentiation, concomitantly depleting the stem cell pool and destabilizing the balance between differentiated trophoblast cell types in the placenta. Warnings about embryonic toxicity in rats in the inserts of two commonly prescribed PPARγ agonists, Avandia (rosi) and Actos (pioglitazone), may reflect similar phenomena. In contrast, short-term administration of acute doses of rosi to pregnant rats during midgestation or chronic exposure of pregnant mice to moderate doses of rosi was harmless [29, 30], as were anecdotal incidents in which pregnant women were accidentally exposed to the drug [31, 32].

The functions of PPARγ in trophoblast differentiation have been simulated in several in vitro systems. For example, stimulation of primary human term trophoblasts by PPARγ agonists enhanced their differentiation into multinucleated syncytiotrophoblasts, in agreement with the critical role of PPARγ in syncytium formation in the mouse labyrinth [33]. In TS cells, the association of PPARγ with trophoblast differentiation is manifested in its dramatic induction during transition from the undifferentiated to the differentiated state [34]. This pattern demonstrates that PPARγ is integral to the process of trophoblast differentiation and pinpoints TS cells as an ideal platform for studying the placental functions of PPARγ. On this front, we recently established *Pparg*-null TS cell lines, whose analysis is currently underway [35].

2.2. PPARγ and trophoblast metabolism

The established roles of PPARγ in systemic and cellular energy metabolism and the importance of trophoblast metabolism for embryonic development raised the plausible hypothesis that PPARγ might regulate metabolic functions of trophoblasts. This idea was strongly supported by the near-complete absence of lipid droplets from the fetal vessel-proximal trophoblast layer of *Pparg*-null placentas as opposed to their WT counterparts, in which these droplets are abundant [12]. Moreover, PPARγ and RXR agonists synergistically stimulate lipid uptake in both cultured trophoblasts in vitro and whole placentas in vivo [28, 36]. These processes are associated with the upregulation of CD36, FABPpm, fatty acid transport proteins 1 and 4 (*Fatp1, Fatp4*), and the lipid droplet proteins adipophilin, S3-12, and MLDP [28, 36]. Thus, PPARγ is an important regulator of lipid dynamics in trophoblasts.

Hypoxia of trophoblasts due to hypoperfusion of the placental bed is a common complication in human pregnancy. Interestingly, agonist-mediated stimulation of PPARγ protects trophoblasts from an acute, but not a long-term apoptotic response to hypoxia [37]. Potential mechanisms underlying this protective effect include PPARγ-dependent

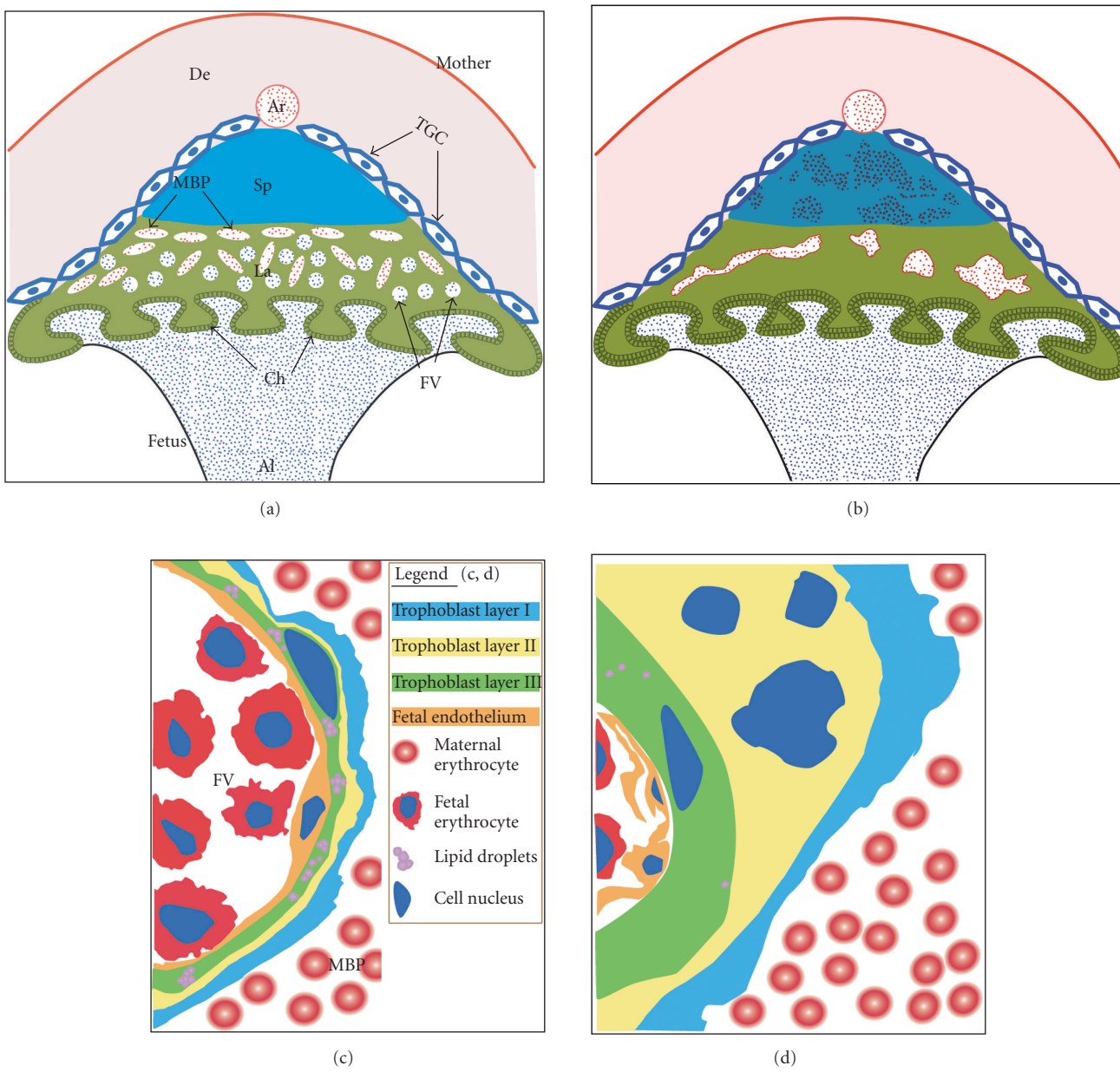


Figure 2: Schematic representation of the *Pparg*-null phenotype. (a) WT placenta. Al: allantois; Ar: maternal artery; Ch: chorion; De: decidua; FV: fetal blood vessels; La: labyrinth; MBP: maternal blood pools; Sp: spongiotrophoblast; TGC: trophoblast giant cells. (b) *Pparg*-null placenta. Corresponding structures are as in (a). Differences of note are marked erythrophagocytosis by spongiotrophoblast cells (red speckles), absence of fetal vessels and breakdown of the maternal blood pools in the labyrinth, and thickening of the chorion. (c,d) Ultrastructural features of WT and *Pparg*-null hemochorial barriers (based on [12]). See legend in (c) for identity of major features. Differences include thickening of the three trophoblast layers, near elimination of lipid droplets in layer III, and loosening of the tight adherence between the trophoblast (green) and fetal endothelium (orange).

differentiation of cytotrophoblasts to syncytiotrophoblasts, which are more resistant to hypoxic death, or direct inhibition of apoptotic pathways by PPARγ.

2.3. Other PPARγ functions in trophoblasts

In addition to the role of PPARγ in trophoblast differentiation and metabolism, it appears to contribute to specialized functions of trophoblasts. One of these unique functions is invasion of the endometrium. The strong coexpression of PPARγ and its obligatory RXRα partner in extravillous cytotrophoblasts at the maternal-fetal interface of human embryos suggested that PPARγ might regulate the invasive functions of trophoblasts. The ability of PPARγ and RXR agonists to inhibit matrigel invasion by both primary and transformed trophoblasts, and the enhancement of invasion by PPARγ and RXR antagonists, supported this hypothesis and implicated PPARγ as a negative regulator of the process

[38, 39]. This activity has been correlated to a 3-fold decrease in the expression of pregnancy-associated plasma protein A (PAPP-A)—a protease essential for maturation of the pro-invasive IGF2—and to a 3-fold induction of Interleukin-1β [40].

Another critical function of trophoblasts is the secretion of reproductive hormones, such as placental lactogens (PL) and choriogonadotropin (hCG). Studies in primary human trophoblasts showed that PPARγ and RXR agonists stimulate hCG and hPL production, and that PPARγ-RXRα heterodimers directly activate hCGβ *via* a PPAR-response element (PPRE) in its promoter [33, 38]. These findings suggest that PPARγ functions extend to trophoblast-specific processes beyond cell differentiation, metabolism, and motility.

2.4. Placental PPARγ target genes

PPARs are transcription factors, and as such, their raison d'être is to regulate the expression of target genes. Identification of these targets is therefore fundamental for determining the biological functions of PPARs. Two primary philosophies underlie target gene identification. The first is a candidate gene approach, which involves hypothesis-driven testing of genes that make plausible targets based either on their established regulation by PPARs in other tissues or on their known relationship to PPAR-regulated processes; trophoblast targets of PPARs found via this approach are described throughout this review in relation to their biological context. The second approach is discovery-based, and involves unbiased, transcriptome-wide screening for target genes based on genetic, pharmacological, and biochemical criteria. The strength of this strategy lies in its ability to break ground and identify targets whose regulation by PPARs would not be otherwise hypothesized.

The identification of *Muc1* as a PPARγ target gene in trophoblasts by subtraction of cDNA from WT versus *Pparg*-null placentas has proven the power of the latter approach to unearth unexpected targets [34]. *Muc1* is very tightly regulated by PPARγ, and its expression is lost in both *Pparg*-null and *Rxra*-null placentas and is upregulated by PPARγ agonists in both differentiated TS cells and whole WT placentas [28, 34]. The *Muc1* protein localizes to apical labyrinthine trophoblasts surrounding maternal blood pools, analogous to its luminal localization on simple secretory epithelia, such as those that abut milk or salivary ducts [34]. This spatial pattern invokes unanticipated anatomical and functional analogies between trophoblasts and prototypic luminal epithelia, raising the provocative idea that some of the placental functions of PPARγ are a carryover from more ancient functions in classical epithelia. However, unlike *Pparg*, *Muc1* is not essential for placental development and its deficiency leads at worst to a mild dilation of the maternal blood pools in the labyrinth [34]. This benign phenotype indicates that other target genes must account for the essential placental functions of PPARγ. Our ongoing microarray-based screens start to uncover new PPARγ targets that may account for these functions [35].

In addition to their prospect in illuminating PPAR functions, new target genes provide novel templates for studying the details of native gene regulation by PPARs. Our studies of the *Muc1* promoter provide an excellent example for the unique insights that such an approach can provide over the study of synthetic promoters or isolated response elements. A proximal *Muc1* promoter fragment responds robustly and in an RXRα-dependent manner to PPARγ and rosi, yet unlike most previously studied PPAR targets, let alone synthetic ones, is entirely refractory to PPARα and PPARδ [34]. Detailed mutation analyses reveal a weak PPRE in the proximal part of the *Muc1* promoter that acts as a basal silencer, and whose derepression by PPARγ is required for robust and specific induction of *Muc1* by an upstream, non-PPAR-binding enhancer [34]. This level of detail reveals previously unappreciated layers of specificity and intricacy underlying the regulation of real-life targets by PPARγ.

2.5. PPARγ and the placenta-heart axis

Analysis of *Pparg*-null embryos unexpectedly found accelerated cardiomyocyte differentiation and thinning of the ventricular wall [12, 41]. This observation was intriguing because at that developmental stage *Pparg* is expressed nowhere but in the placenta. Consistent with this expression pattern, complete reversal of the cardiac defects in *Pparg*-null tetraploid chimeras confirmed that these anomalies are secondary to the placental defects [12]. This result invoked a previously unappreciated dependence of early heart development on placental integrity [12]. How placental *Pparg* deficiency underlies cardiac malformation is currently unclear and could involve generalized nutritional, vascular, or metabolic deficiencies, hypoxia, or a deficiency for placenta-derived factors. However, similar cardiac defects are often observed in association with placental anomalies (reviewed in [42]), and the "placenta-heart axis" has been since reinforced in *p38a*-null embryos, which phenocopy the *Pparg*-null placental and cardiac defects and are similarly rescued by tetraploid chimeras [11]. Therefore, myocardial failure is likely a general attribute of placental insufficiency and not a specific consequence of PPARγ mutation.

3. PPARδ

As in the case of PPARγ, the finding that *Ppard*-null embryos succumb to lethal placental defects was also unexpected [43, 44]. The first *Ppard*-null mouse strain reported was generated by truncating the gene a mere 60 amino acids from its C-terminus (*Ppard*-ΔC60), leaving the entire DNA-binding domain and most of the ligand-binding domain intact [45]. While this allele is likely a hypomorph, the authors reported significantly smaller size and lower survival rates of the original F2 homozygotes for this allele, which they have overcome by outbreeding and consecutive mating of the survivors [45]. In contrast, mice in which PPARδ was inactivated by CRE/*loxP*-mediated truncation of the N-terminal half of the DNA-binding domain and frame-shifting of the remaining 3' part of *Ppard* mRNA exhibited overwhelming embryonic lethality and placental defects, as detailed in Section 3.1 [43]. Nevertheless, a few homozygous-null mice survived gestation thanks to a complex influence of genet-

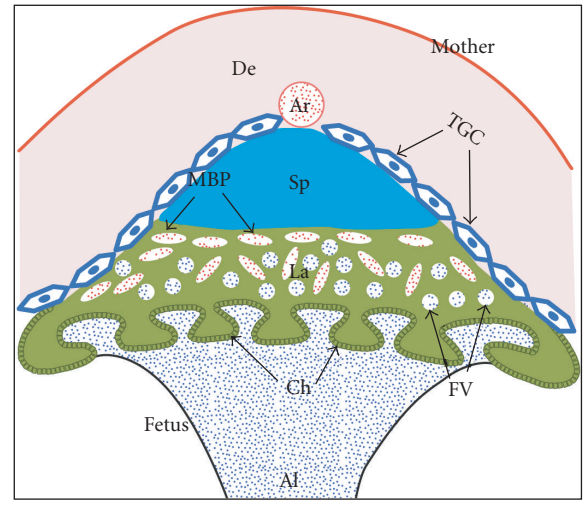
ics and maternal physiology (see Section 3.2). Two other null configurations, one with *lacZ* insertion into the DNA-binding domain of PPARδ [46, 47] and another that replaced the DNA-binding domain with PGK-neo [44], yielded identical lethality and placental defects, confirming that PPARδ is indeed essential for placental function.

3.1. PPARδ in placental development and integrity

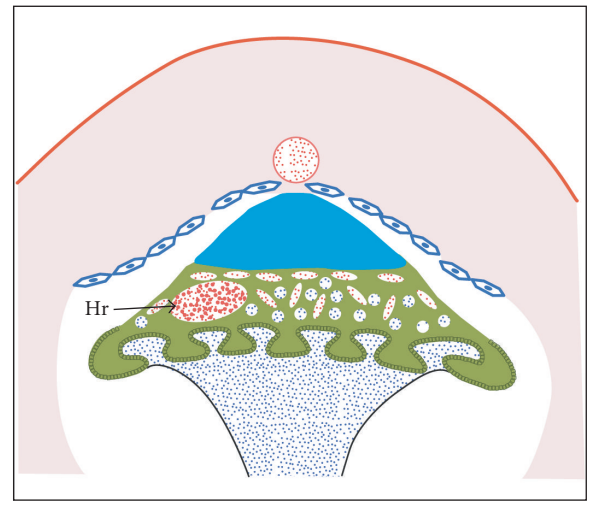
Lethality and sub-Mendelian ratios of *Ppard*-null embryos are observed from E9.5–10.5 onward. Rare null embryos surviving beyond that stage typically exhibit severe flooding of maternal blood into the placental and embryionic space, are significantly smaller than their WT and heterozygous siblings, and the few that survive to birth are markedly runt [43, 44]. Still, none dies after birth and all thrive and become generally healthy and fertile adults, despite remaining slightly smaller than their *Ppard* sufficient counterparts [43]. The combination of strictly prenatal mortality, growth restriction, and abundant expression of *Ppard* in the placenta points to critical defects in extraembryonic tissue.

From as early as E8.5 onward, *Ppard*-null embryos and placentas are significantly smaller than their littermates [43, 44]. All placental compartments are smaller, including the labyrinth, the spongiotrophoblast, and the giant cell layer. The latter is severely thinner and discontinuous, with cells that do not attain the maximal size typical of WT giant cells (43, 44). This compromise in giant cell size and continuity likely underlies the observed loosening of the normally tight placenta-decidua interface and the inability to retrieve *Ppard*-null specimens from E9.5 onward without substantial detachment of placentas from the deciduas [43]. In contrast, while the labyrinth is smaller, its vascular structure is fully elaborated, clearly distinguishing the *Ppard*-null from the *Pparg*-null placental phenotype [43]. These features are summarized schematically in Figure 3.

Consistent with the implicated role of PPARδ in giant cell differentiationin vivo, studies of the trophoblast cell line Rcho-1 have unequivocally demonstrated that PPARδ is crucial for giant cell differentiation in vitro [44]. Agonist-mediated stimulation of PPARδ dramatically accelerated differentiation of Rcho-1 cells into giant cells, whereas siRNA-mediated knockdown of PPARδ severely inhibited the process. PPARδ was necessary and sufficient for suppression of Id-2, which inhibits giant cell differentiation, and for upregulation of I-mfa, which promotes giant cell differentiation by antagonizing the bHLH transcription factor Mash-2. Interestingly, in trophoblasts, just like in keratinocytes, PPARδ upregulates the expression of two key nodes in the PI3 kinase (PI3K)/Akt signaling pathway: PDK1 and ILK. These, in turn, activate Akt by phosphorylating two residues: Thr308 and Ser473. Activation of this pathway is critical for the ability of PPARδ to accelerate giant cell differentiation, and a synthetic PI3K inhibitor completely reversed upregulation of PL-1, downregulation of Id-2, and giant cell formation. However, additional pathways are at play downstream of PPARδ, as evident in the insensitivity to PI3K inhibition of PPARδ-dependent I-mfa activation.



(a)



(b)

Figure 3: Schematic representation of the *Ppard*-null phenotype. (a) *WT* placenta (similar to Figure 2(a)). (b) *Ppard*-null placenta. Hr: hemorrhage; for all other abbreviations see the legend for Figure 2. Notable differences include smaller and discontinuous giant cells, reduced size of the entire placenta and loosening of its attachment to the decidua, and sporadic severe hemorrhages at various locations in or around the placenta.

3.2. Genetic and maternal modifications of the Ppard-null phenotype

Surprisingly, all *Ppard* deficient alleles exhibit highly variable penetrance of both the placental phenotype and lethality itself. Our early studies of *Ppard*-null mice encountered a clear maternal effect on the fate of *Ppard*-null embryos. These studies were carried out on either a pure 129/SvJae *129* background or a segregating F2, F3, and F4-C57BL/6J [*B6*]: *129* background, in which the vast majority of homozygous null

embryos die during gestation [43]. However, 2–5% of *129-Ppard*-null mice and 10–15% of *B6*: *129-Ppard*-null mice survived to parturition. These rare survival events were not randomly distributed. First, litters with multiple null pups (up to 4 in one litter) were frequently observed [43, 47]. Second, all survival cases occurred in first-time pregnancies, none recurring in the same breeding pair. Third, survival was not heritable in these cases, that is, null mice were fully fertile, but never gave birth to *Ppard*-null progeny when crossed with $Ppard^{+/-}$ or $Ppard^{-/-}$ mates. This substantial deviation from random distribution suggested that survival on these genetic backgrounds is modified primarily by maternal conditions rather than genetics. A hypothetical example of such conditions is slow immune attack of first-time mothers on embryos with breached immune privilege.

Notwithstanding maternal effects, the *Ppard*-null phenotype is also clearly subject to genetic modification. Peters et al. alluded to poor survival of the initial batch of homozygous *Ppard*-ΔC60 mice and the complete resolution of this problem by an additional backcross of F1 mice with inbred C57BL/6N mates, which yielded normal Mendelian distribution of the progeny starting at F3 [45]. Similarly, Nadra et al. reported very low survival rates of outbred *B6:129-Ppard*-null mice, which was eventually overcome by intercrossing rare surviving mutants [44]. Our work in progress sheds further light on the effects of genetic modifiers on the *Ppard*-null phenotype. First, repetitive backcrosses onto *B6* completely obliterates survival of mutants beyond E9.5, indicating that *129*-specific alleles allow mutants to survive 1-2 days longer than *B6* alleles and are more permissive towards the survival of *Ppard*-null embryos to term [47]. Second, when $B6{:}Ppard^{+/-}$ mice are backcrossed onto an *FVB/NJ* (*FVB*) background, intercrosses of the heterozygous F1 generation result in survival of ∼15% of the expected *Ppard*-null progeny [47]. On this background, survival of F2 *FVB:B6-Ppard-null* mice is evenly distributed and not limited to first time pregnancies. Thus, *FVB* alleles are permissive for survival of *Ppard*-null embryos, yet in a substantially different way than the *129* or *B6:129* backgrounds. Third, survival of FVB:B6 *Ppard*-null embryos is heritable, and multigenerational intercrosses of F2-*FVB:B6-Ppard*-null parent pairs and their progeny led to the establishment of a semistable stock of viable *Ppard*-null mice [47]. This stock has reached a reproductive plateau by F4, and now consistently yields survival of approximately 50% of the *Ppard*-null progeny. Further inspection reveals that all progeny survive to E10.0, when approximately half of the litter develops abnormal histological features at the placenta-decidua interface and succumbs to transplacental infiltration of maternal blood and fatal hemorrhaging and necrosis. In contrast, the placentas of viable *Ppard*-null embryos from this stock are broadly normal. At present, it is not clear whether this sharp partition represents a stochastically incomplete penetrance or rather a discrete genetic or epigenetic modifier that is inherited by only 50% of the progeny.

In conclusion, placental PPARδ regulates essential processes, which are highly interactive with the genetic and maternal environments. Further studies of the *Ppard*-null phenotype, its response to experimentally defined maternal variables, and identification of genes that modify its nature and outcomes should yield new insights into the biology of both PPARδ and the placenta.

4. TRANSCRIPTIONAL PARTNERS OF PPARS

The ability of PPARs to bind DNA and activate transcription depends strictly on heterodimerization with retinoid-X receptors (RXRs) [48]. In addition, diverse transcriptional coactivator proteins are indispensable for transcriptional activation by PPAR-RXR heterodimers. These interdependencies imply that both RXRs and relevant coactivators should be essential for placental functions of PPARs and their deficiencies should yield comparable phenotypes.

4.1. RXRs

RXRα is the major RXR isoform in the placenta [49], and its deficiency is therefore expected to recapitulate lethal placental defects of *Pparg*-null and *Ppard*-null embryos. Indeed, *Rxra*-null placentas exhibit multiple defects, some of which are similar to defects in *Pparg*-null placentas, including the following: (a) incomplete compaction of labyrinthine trophoblasts, (b) disruption of the basement membrane and the tight contact between labyrinthine trophoblasts and infiltrating fetal endothelium, (c) a marked reduction in lipid droplet content of labyrinthine trophoblasts, and (d) maternal hematomas at the junctional zone [50]. Other defects, such as partial disorganization of the labyrinthine zone, invasion of spongiotrophoblast cells into the labyrinth, and reduced number of glycogen cells, are not an obvious extrapolation of either the *Pparg*-null or the *Ppard*-null phenotype.

Still, *Rxra*-null embryos die between E12.5 and E16.5 [51, 52], and the aforementioned placental anomalies are observed later than the lethal endpoints of either PPAR deficiency. Therefore, these defects can represent at best an incomplete knockdown of PPARγ and δ activities. This milder phenotype is apparently rooted in functional redundancy with RXRβ, as evident in the markedly accelerated and exacerbated *Rxra*/*Rxrb* double null phenotype [53]. *Rxra*/*b* double null embryos die at E9.5 while exhibiting a combination of failed placental vascularization, which is a hallmark of *Pparg* deficiency, and severe placenta-decidua detachment, as in *Ppard*-null embryos. This phenotype suggests that although RXRα is the primary PPAR partner in the placenta, RXRβ provides a redundant, albeit incomplete backup for PPAR function in the placenta.

The most conspicuous phenotype of *Rxra*-null embryos is severe thinning and incomplete septation of the cardiac ventricles, which is the likely cause of their death [51, 52]. This phenotype is non-cardiomyocyte-autonomous [54] and has been successfully recapitulated by ablation of retinoic acid signaling in the epicardium [55]. Consequently, its relationship to the placental defects has never been investigated. Nevertheless, the proven dependence of myocardial hypoplasia on placental defects in *Pparg*-null embryos raises the need to examine whether at least some aspects of the cardiac *Rxra*-null phenotype can be traced back to placental defects.

4.2. CoActivators

Among the large array of cofactors that mediate transactivation functions of PPAR-RXR heterodimers, two stand out in the context of placental functions: PBP/DRIP205/TRAP220 (official gene name: *Pparbp*) and PRIP/AIB3/RAP250 (official name: *Ncoa6*). Three teams knocked out *Pparbp* and found that homozygous null embryos die at E11.5 concomitant with growth restriction and myocardial hypoplasia [56–58]. One team described placental defects that included poor compaction of labyrinthine trophoblasts, reduced vascularization, and phagocytosis of maternal erythrocytes, recapitulating multiple histological and ultrastructural features of *Pparg*-null placentas [56]. These observations suggested that PPARBP coactivates essential developmental targets of PPARγ-RXRα/β heterodimers in the placenta, and the later lethality of these mutants suggested partial redundancy with other coactivators. A second team saw no overt morphological defects in *Pparbp*-null placentas, but found that tetraploid chimeras postponed lethality of the mutants from E11.5 to E13.5, proving that the homozygous-null embryos nevertheless die due to placental defects [57]. Interestingly, tetraploid chimeras did not rescue the cardiac defects of *Pparbp*-null mice, demonstrating that these defects evolve irrespective of the placental problems, unlike in the case of *Pparg* deficiency.

Three teams of investigators generated and analyzed different *Ncoa6*-null mouse strains that exhibited different grades of phenotypic severity [59–61]. One team targeted *Ncoa6* by deleting exons 4 through 7 [59]. Homozygous-null embryos died around E10.0, preceded by substantial growth restriction, severe myocardial thinning, and a series of placental defects that closely resembled those of *Pparg*-null placentas. These included (a) failed vascularization of the labyrinth, (b) poor compaction of syncytiotrophoblasts, (c) dilation and rupture of the maternal blood pools, and (d) erythrophagocytosis in the junctional zone. An additional placental phenotype not shared with *Pparg*-null placentas was thickening of the giant cell layer alongside thinning of the spongiotrophoblast and the labyrinthine zones [59]. These overall similarities indicated that *Ncoa6* is critical for the essential transcriptional functions of PPARγ and perhaps additional transcription factors in the placenta and that *Ncoa6* deficiency is not compensated for by genetic redundancy. The other two teams interrupted the gene downstream of exon 6, and reported undetectable levels of *Ncoa6* gene products, but a significantly milder phenotype [60, 61], which suggested that both configurations are functional hypomorphs. Homozygous-targeted embryos for these alleles died around E13.5 and exhibited myocardial hypoplasia and placental defects that included a thin spongiotrophoblast layer, ectopic spongiotrophoblasts within the labyrinth, reduced vascularization of the labyrinth, and stasis and necrosis in the junctional zone [60, 61]. Interestingly, these features are highly reminiscent of the *Rxra*-null phenotype, suggesting that they indeed reflect incomplete loss of *Pparg* function.

While the phenotypes of *Ncoa6* and *Pparbp*-null mice pinpoint the two as essential coactivators of PPARγ-RXRα/β transcription complexes in the developing placenta, this is by no means the complete inventory of cofactors that are crucial for placental functions of PPARs. First, no cofactor knockout has so far yielded a *Ppard*-null-like phenotype. Second, possible roles of cofactors that have not yielded clear placental phenotypes cannot be ruled out. For example, mice deficient for either CBP or p300 die during early gestation [62–64], and because extraembryonic tissues were not carefully examined in these mutants, placental defects are still a strong possibility. Another complication is presented by families of homologous cofactors with a high potential for functional redundancies, such as the p160 coactivators SRC-1, TIF2, and ACTR/SRC-3 or the PGC-1 family, that is, PGC-1α, PGC-1β, and PRC. While single deficiencies for any of these cofactors are not embryonic lethal, therefore precluding serious placental defects, one should keep in mind that compensation by remaining family members may well be at play.

5. CONCLUSIONS AND PROSPECTS

As detailed in this review, PPARγ and PPARδ play nonredundant roles in placental development and physiology. PPARγ is a key regulator of trophoblast differentiation and metabolism, PPARδ is essential for giant cell function and placental integrity, and their coreceptors RXRα and β are instrumental for the execution of these functions. At least two transcriptional coactivators, PPARBP and NCOA6, are critical for essential functions of PPARγ in the placenta, as deduced from the *Pparg*-null-like phenotype of their deficiencies, and additional cofactors are likely crucial for those of PPARδ.

Still, the network of signals upstream, alongside, and downstream of PPARγ and PPARδ is far from elucidated. Several PPAR targets have been identified in trophoblasts, providing initial mechanistic insights into PPAR function in the placenta. However, the discovery of as many new target genes will be indispensable for fully deciphering these functions. Another important effort should be to determine the various regulators that control or modify PPAR expression and activity in trophoblasts. These include, but are not limited to upstream transcriptional regulators, molecules that control the stability of PPAR gene products, posttranslational modifications that alter the functions of PPARs, RXRs, or their cofactors, and the production and dissemination of endogenous ligands. Many of these processes may constitute key regulatory nodes in placental physiology. In addition, PPAR-specific features, such as the identity of genes that modify the outcomes of PPARδ deficiency, would provide invaluable insights.

Finally, identifying compelling similarities between the *Ppar*-null placental phenotypes and published descriptions of targeted genes with previously unknown connections presents a complementary approach for identifying critical nodes in placental PPAR signaling. Such a strategy has been widely successful in identifying a plethora of epistatic relationships in lower eukaryotes such as yeast, nematodes, and flies, and more recently in identifying novel SHH signaling components in mice [65]. Because placental defects are among the earliest roadblocks in the development of many gene-targeted embryos, such opportunities abound. For ex-

ample, the published analyses of single and compound keratin 8 (*mK8*), *mK18*, and *mK19* knockouts reveal remarkable similarities to the *Ppard*-null placental phenotype [66–69]. Similarly, the placental and cardiac phenotypes of αV- and β8-integrins, p38α, *JunB*, and *Fra1* knockouts are strikingly similar to those of *Pparg*-null embryos [9–11, 70, 71]. Integrating studies of these genes and their corresponding pathways into the functional studies of PPARs and their regulators, associated factors, and transcriptional targets should provide further insights into the mode by which PPAR signaling networks regulate placental development.

ACKNOWLEDGMENTS

The authors thank Barbara Kaegi and Jennifer Torrance for expert graphics and Pat Cherry for administrative assistance. Y. Barak is supported by NIH Grant R01HD044103 and by a grant from The March of Dimes. Y. Sadovsky is supported by NIH Grant R01ES011597.

REFERENCES

[1] A. Ornoy, J. Salamon-Arnon, Z. Ben-Zur, and G. Kohn, "Placental findings in spontaneous abortions and stillbirths," *Teratology*, vol. 24, no. 3, pp. 243–252, 1981.

[2] C. Krebs, L. M. Macara, R. Leiser, A. W. Bowman, I. A. Greer, and J. C. P. Kingdom, "Intrauterine growth restriction with absent end-diastolic flow velocity in the umbilical artery is associated with maldevelopment of the placental terminal villous tree," *American Journal of Obstetrics and Gynecology*, vol. 175, no. 6, pp. 1534–1542, 1996.

[3] Y. Zhou, C. H. Damsky, K. Chiu, J. M. Roberts, and S. J. Fisher, "Preeclampsia is associated with abnormal expression of adhesion molecules by invasive cytotrophoblasts," *Journal of Clinical Investigation*, vol. 91, no. 3, pp. 950–960, 1993.

[4] J. C. Cross, "How to make a placenta: mechanisms of trophoblast cell differentiation in mice—a review," *Placenta*, vol. 26, supplement 1, pp. S3–S9, 2005.

[5] D. G. Simmons and J. C. Cross, "Determinants of trophoblast lineage and cell subtype specification in the mouse placenta," *Developmental Biology*, vol. 284, no. 1, pp. 12–24, 2005.

[6] F. Guillemot, A. Nagy, A. Auerbach, J. Rossant, and A. L. Joyner, "Essential role of Mash-2 in extraembryonic development," *Nature*, vol. 371, no. 6495, pp. 333–336, 1994.

[7] J. Luo, R. Sladek, J.-A. Bader, A. Matthyssen, J. Rossant, and V. Giguère, "Placental abnormalities in mouse embryos lacking the orphan nuclear receptor ERR-β," *Nature*, vol. 388, no. 6644, pp. 778–782, 1997.

[8] H. Yamamoto, M. L. Flannery, S. Kupriyanov, et al., "Defective trophoblast function in mice with a targeted mutation of Ets2," *Genes and Development*, vol. 12, no. 9, pp. 1315–1326, 1998.

[9] M. Schorpp-Kistner, Z.-Q. Wang, P. Angel, and E. F. Wagner, "JunB is essential for mammalian placentation," *EMBO Journal*, vol. 18, no. 4, pp. 934–948, 1999.

[10] M. Schreiber, Z.-Q. Wang, W. Jochum, I. Fetka, C. Elliott, and E. F. Wagner, "Placental vascularisation requires the AP-1 component Fra1," *Development*, vol. 127, no. 22, pp. 4937–4948, 2000.

[11] R. H. Adams, A. Porras, G. Alonso, et al., "Essential role of p38α MAP kinase in placental but not embryonic cardiovascular development," *Molecular Cell*, vol. 6, no. 1, pp. 109–116, 2000.

[12] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[13] C. Schmidt, F. Bladt, S. Goedecke, et al., "Scatter factor/hepatocyte growth factor is essential for liver development," *Nature*, vol. 373, no. 6516, pp. 699–702, 1995.

[14] Y. Uehara, O. Minowa, C. Mori, et al., "Placental defect and embryonic lethality in mice lacking hepatocyte growth factor/scatter factor," *Nature*, vol. 373, no. 6516, pp. 702–705, 1995.

[15] D. W. Threadgill, A. A. Dlugosz, L. A. Hansen, et al., "Targeted disruption of mouse EGF receptor: effect of genetic background on mutant phenotype," *Science*, vol. 269, no. 5221, pp. 230–234, 1995.

[16] M. Sibilia and E. F. Wagner, "Strain-dependent epithelial defects in mice lacking the EGF receptor," *Science*, vol. 269, no. 5221, pp. 234–238, 1995.

[17] C. B. Ware, M. C. Horowitz, B. R. Renshaw, et al., "Targeted disruption of the low-affinity leukemia inhibitory factor receptor gene causes placental, skeletal, neural and metabolic defects and results in perinatal death," *Development*, vol. 121, no. 5, pp. 1283–1299, 1995.

[18] R. Ohlsson, P. Falck, M. Hellström, et al., "PDGFB regulates the development of the labyrinthine layer of the mouse fetal placenta," *Developmental Biology*, vol. 212, no. 1, pp. 124–136, 1999.

[19] S. J. Monkley, S. J. Delaney, D. J. Pennisi, J. H. Christiansen, and B. J. Wainwright, "Targeted disruption of the Wnt2 gene results in placentation defects," *Development*, vol. 122, no. 11, pp. 3343–3353, 1996.

[20] T.-O. Ishikawa, Y. Tamai, A. M. Zorn, et al., "Mouse Wnt receptor gene Fzd5 is essential for yolk sac and placental angiogenesis," *Development*, vol. 128, no. 1, pp. 25–33, 2001.

[21] S. Tanaka, T. Kunath, A.-K. Hadjantonakis, A. Nagy, and J. Rossant, "Promotion to trophoblast stem cell proliferation by FGF4," *Science*, vol. 282, no. 5396, pp. 2072–2075, 1998.

[22] A. Erlebacher, K. A. Price, and L. H. Glimcher, "Maintenance of mouse trophoblast stem cell proliferation by TGF-β/activin," *Developmental Biology*, vol. 275, no. 1, pp. 158–169, 2004.

[23] J. Yan, S. Tanaka, M. Oda, T. Makino, J. Ohgane, and K. Shiota, "Retinoic acid promotes differentiation of trophoblast stem cells to a giant cell fate," *Developmental Biology*, vol. 235, no. 2, pp. 422–432, 2001.

[24] A. Nagy, E. Gocza, E. Merentes Diaz, et al., "Embryonic stem cells alone are able to support fetal development in the mouse," *Development*, vol. 110, no. 3, pp. 815–821, 1990.

[25] S. Z. Duan, C. Y. Ivashchenko, S. E. Whitesall, et al., "Hypotension, lipodystrophy, and insulin resistance in generalized PPARγ-deficient mice rescued from embryonic lethality," *Journal of Clinical Investigation*, vol. 117, no. 3, pp. 812–822, 2007.

[26] S. Kim and Y. Barak, unpublished data.

[27] D. P. Hewitt, P. J. Mark, and B. J. Waddell, "Placental expression of peroxisome proliferator-activated receptors in rat pregnancy and the effect of increased glucocorticoid exposure," *Biology of Reproduction*, vol. 74, no. 1, pp. 23–28, 2006.

[28] W. T. Schaiff, F. F. Knapp Jr., Y. Barak, T. Biron-Shental, D. M. Nelson, and Y. Sadovsky, "Ligand-activated PPARγ alters placental morphology and placental fatty acid uptake in mice,"

Endocrinology, vol. 148, no. 8, pp. 3625–3634, 2007.

[29] R. Asami-Miyagishi, S. Iseki, M. Usui, K. Uchida, H. Kubo, and I. Morita, "Expression and function of PPARγ in rat placental development," *Biochemical and Biophysical Research Communications*, vol. 315, no. 2, pp. 497–501, 2004.

[30] D. B. Klinkner, H. J. Lim, E. Y. Strawn Jr., K. T. Oldham, and T. L. Sander, "An in vivo murine model of rosiglitazone use in pregnancy," *Fertility and Sterility*, vol. 86, no. 4, supplement 1, pp. 1074–1079, 2006.

[31] F. Yaris, E. Yaris, M. Kadioglu, C. Ulku, M. Kesim, and N. I. Kalyoncu, "Normal pregnancy outcome following inadvertent exposure to rosiglitazone, gliclazide, and atorvastatin in a diabetic and hypertensive woman," *Reproductive Toxicology*, vol. 18, no. 4, pp. 619–621, 2004.

[32] N. I. Kalyoncu, F. Yaris, C. Ulku, et al., "A case of rosiglitazone exposure in the second trimester of pregnancy," *Reproductive Toxicology*, vol. 19, no. 4, pp. 563–564, 2005.

[33] W. T. Schaiff, M. G. Carlson, S. D. Smith, R. Levy, D. M. Nelson, and Y. Sadovsky, "Peroxisome proliferator-activated receptor-γ modulates differentiation of human trophoblast in a ligand-specific manner," *Journal of Clinical Endocrinology and Metabolism*, vol. 85, no. 10, pp. 3874–3881, 2000.

[34] T. Shalom-Barak, J. M. Nicholas, Y. Wang, et al., "Peroxisome proliferator-activated receptor γ controls Muc1 transcription in trophoblasts," *Molecular and Cellular Biology*, vol. 24, no. 24, pp. 10661–10669, 2004.

[35] X. Zhang, T. Shalom-Barak, W. T. Schaiff, Y. Sadovsky, and Y. Barak, MS in preparation.

[36] W. T. Schaiff, I. Bildirici, M. Cheong, P. L. Chern, D. M. Nelson, and Y. Sadovsky, "Peroxisome proliferator-activated receptor-γ and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 7, pp. 4267–4275, 2005.

[37] U. Elchalal, R. G. Humphrey, S. D. Smith, C. Hu, Y. Sadovsky, and D. M. Nelson, "Troglitazone attenuates hypoxia-induced injury in cultured term human trophoblasts," *American Journal of Obstetrics and Gynecology*, vol. 191, no. 6, pp. 2154–2159, 2004.

[38] A. Tarrade, K. Schoonjans, J. Guibourdenche, et al., "PPARγ/RXRα heterodimers are involved in human CGβ synthesis and human trophoblast differentiation," *Endocrinology*, vol. 142, no. 10, pp. 4504–4514, 2001.

[39] L. Pavan, A. Tarrade, A. Hermouet, et al., "Human invasive trophoblasts transformed with simian virus 40 provide a new tool to study the role of PPARγ in cell invasion process," *Carcinogenesis*, vol. 24, no. 8, pp. 1325–1336, 2003.

[40] T. Fournier, K. Handschuh, V. Tsatsaris, and D. Evain-Brion, "Involvement of PPARγ in human trophoblast invasion," *Placenta*, vol. 28, supplement, pp. S76–S81, 2007.

[41] N. Kubota, Y. Terauchi, H. Miki, et al., "PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance," *Molecular Cell*, vol. 4, no. 4, pp. 597–609, 1999.

[42] M. Hemberger and J. C. Cross, "Genes governing placental development," *Trends in Endocrinology and Metabolism*, vol. 12, no. 4, pp. 162–168, 2001.

[43] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[44] K. Nadra, S. I. Anghel, E. Joye, et al., "Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor β/δ," *Molecular and Cellular Biology*, vol. 26, no. 8, pp. 3266–3281, 2006.

[45] J. M. Peters, S. S. T. Lee, W. Li, et al., "Growth, adipose, brain, and skin alterations resulting from targeted disruption of the mouse peroxisome proliferator-activated receptor $\beta(\delta)$," *Molecular and Cellular Biology*, vol. 20, no. 14, pp. 5119–5128, 2000.

[46] A. Chawla, C.-H. Lee, Y. Barak, et al., "PPARδ is a very low-density lipoprotein sensor in macrophages," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 3, pp. 1268–1273, 2003.

[47] Y. Barak, unpublished data.

[48] S. A. Kliewer, K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans, "Convergence of 9-cis retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors," *Nature*, vol. 358, no. 6389, pp. 771–774, 1992.

[49] V. Sapin, S. J. Ward, S. Bronner, P. Chambon, and P. Dollé, "Differential expression of transcripts encoding retinoid binding proteins and retinoic acid receptors during placentation of the mouse," *Developmental Dynamics*, vol. 208, no. 2, pp. 199–210, 1997.

[50] V. Sapin, P. Dollé, C. Hindelang, P. Kastner, and P. Chambon, "Defects of the chorioallantoic placenta in mouse RXRα null fetuses," *Developmental Biology*, vol. 191, no. 1, pp. 29–41, 1997.

[51] H. M. Sucov, E. Dyson, C. L. Gumeringer, J. Price, K. R. Chien, and R. M. Evans, "RXRα mutant mice establish a genetic basis for vitamin A signaling in heart morphogenesis," *Genes and Development*, vol. 8, no. 9, pp. 1007–1018, 1994.

[52] P. Kastner, J. M. Grondona, M. Mark, et al., "Genetic analysis of RXRα developmental function: convergence of RXR and RAR signaling pathways in heart and eye morphogenesis," *Cell*, vol. 78, no. 6, pp. 987–1003, 1994.

[53] O. Wendling, P. Chambon, and M. Mark, "Retinoid X receptors are essential for early mouse development Land placentogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 2, pp. 547–551, 1999.

[54] C. M. Tran and H. M. Sucov, "The RXRα gene functions in a non-cell-autonomous manner during mouse cardiac morphogenesis," *Development*, vol. 125, no. 10, pp. 1951–1956, 1998.

[55] T. H.-P. Chen, T.-C. Chang, J.-O. Kang, et al., "Epicardial induction of fetal cardiomyocyte proliferation via a retinoic acid-inducible trophic factor," *Developmental Biology*, vol. 250, no. 1, pp. 198–207, 2002.

[56] Y. Zhu, C. Qi, Y. Jia, J. S. Nye, M. S. Rao, and J. K. Reddy, "Deletion of PBP/PARBP, the gene for nuclear receptor coactivator peroxisome proliferator-activated receptor-binding protein, results in embryonic lethality," *Journal of Biological Chemistry*, vol. 275, no. 20, pp. 14779–14782, 2000.

[57] C. Landles, S. Chalk, J. H. Steel, et al., "The thyroid hormone receptor-associated protein TRAP220 is required at distinct embryonic stages in placental, cardiac, and hepatic development," *Molecular Endocrinology*, vol. 17, no. 12, pp. 2418–2435, 2003.

[58] M. Ito, C.-X. Yuan, H. J. Okano, R. B. Darnell, and R. G. Roeder, "Involvement of the TRAP220 component of the TRAP/SMCC coactivator complex in embryonic development and thyroid hormone action," *Molecular Cell*, vol. 5, no. 4, pp. 683–693, 2000.

[59] S.-Q. Kuang, L. Liao, H. Zhang, et al., "Deletion of the cancer-amplified coactivator AIB3 results in defective placentation and embryonic lethality," *Journal of Biological Chemistry*, vol. 277, no. 47, pp. 45356–45360, 2002.

[60] Y.-J. Zhu, S. E. Crawford, V. Stellmach, et al., "Coactivator PRIP, the peroxisome proliferator-activated receptor-interacting protein, is a modulator of placental, cardiac, hepatic, and embryonic development," *Journal of Biological Chemistry*, vol. 278, no. 3, pp. 1986–1990, 2003.

[61] P. Antonson, G. U. Schuster, L. Wang, et al., "Inactivation of the nuclear receptor coactivator RAP250 in mice results in placental vascular dysfunction," *Molecular and Cellular Biology*, vol. 23, no. 4, pp. 1260–1268, 2003.

[62] T.-P. Yao, S. P. Oh, M. Fuchs, et al., "Gene dosage-dependent embryonic development and proliferation defects in mice lacking the transcriptional integrator p300," *Cell*, vol. 93, no. 3, pp. 361–372, 1998.

[63] Y. Oike, N. Takakura, A. Hata, et al., "Mice homozygous for a truncated form of CREB-binding protein exhibit defects in hematopoiesis and vasculo-angiogenesis," *Blood*, vol. 93, no. 9, pp. 2771–2779, 1999.

[64] A. L. Kung, V. I. Rebel, R. T. Bronson, et al., "Gene dose-dependent control of hematopoiesis and hematologic tumor suppression by CBP," *Genes and Development*, vol. 14, no. 3, pp. 272–277, 2000.

[65] M. J. García-García, J. T. Eggenschwiler, T. Caspary, et al., "Analysis of mouse embryonic patterning and morphogenesis by forward genetics," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 17, pp. 5913–5919, 2005.

[66] H. Baribault, J. Price, K. Miyai, and R. G. Oshima, "Mid-gestational lethality in mice lacking keratin 8," *Genes and Development*, vol. 7, no. 7 A, pp. 1191–1202, 1993.

[67] D. Jaquemar, S. Kupriyanov, M. Wankell, et al., "Keratin 8 protection of placental barrier function," *Journal of Cell Biology*, vol. 161, no. 4, pp. 749–756, 2003.

[68] M. Hesse, T. Franz, Y. Tamai, M. M. Taketo, and T. M. Magin, "Targeted deletion of keratins 18 and 19 leads to trophoblast fragility and early embryonic lethality," *EMBO Journal*, vol. 19, no. 19, pp. 5060–5070, 2000.

[69] Y. Tamai, T.-O. Ishikawa, M. R. Bösl, et al., "Cytokeratins 8 and 19 in the mouse placental development," *Journal of Cell Biology*, vol. 151, no. 3, pp. 563–572, 2000.

[70] B. L. Bader, H. Rayburn, D. Crowley, and R. O. Hynes, "Extensive vasculogenesis, angiogenesis, and organogenesis precede lethality in mice lacking all v integrins," *Cell*, vol. 95, no. 4, pp. 507–519, 1998.

[71] J. Zhu, K. Motejlek, D. Wang, K. Zang, A. Schmidt, and L. F. Reichardt, "β8 Integrins are required for vascular morphogenesis in mouse embryos," *Development*, vol. 129, no. 12, pp. 2891–2903, 2002.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 527048, 14 pages
doi:10.1155/2008/527048

Review Article

PPAR Action in Human Placental Development and Pregnancy and Its Complications

Fritz Wieser, Leslie Waite, Christophe Depoix, and Robert N. Taylor

Department of Gynecology and Obstetrics, Emory University School of Medicine, Atlanta, GA 30322, USA

Correspondence should be addressed to Robert N. Taylor, robert.n.taylor@emory.edu

Received 2 September 2007; Accepted 13 November 2007

Recommended by P. Froment

During pregnancy crucial anatomic, physiologic, and metabolic changes challenge the mother and the fetus. The placenta is a remarkable organ that allows the mother and the fetus to adapt to the new metabolic, immunologic, and angiogenic environment imposed by gestation. One of the physiologic systems that appears to have evolved to sustain this metabolic regulation is mediated by peroxisome proliferator-activated receptors (PPARs). In clinical pregnancy-specific disorders, including preeclampsia, gestational diabetes, and intrauterine growth restriction, aberrant regulation of components of the PPAR system parallels dysregulation of metabolism, inflammation and angiogenesis. This review summarizes current knowledge on the role of PPARs in regulating human trophoblast invasion, early placental development, and also in the physiology of clinical pregnancy and its complications. As increasingly indicated in the literature, pregnancy disorders, such as preeclampsia and gestational diabetes, represent potential targets for treatment with PPAR ligands. With the advent of more specific PPAR agonists that exhibit efficacy in ameliorating metabolic, inflammatory, and angiogenic disturbances, further studies of their application in pregnancy-related diseases are warranted.

1. INTRODUCTION

Peroxisome proliferator-activated receptors (PPARs) are major regulators of lipid and glucose metabolism, inflammation, and angiogenesis [1–6] that allow adaptation of the mother to the nutritional and perfusion requirements of the fetus [3, 7, 8]. PPARs, members of the nuclear hormone receptor superfamily, are ligand-activated transcription factors. The PPAR amino acid sequence can be divided into five modular domains: A/B, C, D, E, and F. Domain E is the ligand binding domain (LBD) and contains a ligand-dependent transcriptional activation function (AF-2). Domain C is the DNA binding domain, formed of two typical zinc fingers. PPARs activate DNA direct repeat response elements by binding as heterodimers with retinoic acid receptor (RXR) partners [9]. There are three PPAR isotypes, PPARα, PPARγ, and PPARβ/δ, that are highly conserved across species, with mouse, rat, and human sequences sharing >80% amino acid homology [6, 10]. The conserved expression of different PPAR and RXR isotypes in both rat and human placentas [11] suggests that these receptors play functional roles in placental lipid transfer and homeostasis. PPARα has a wide distribution and is prominent in tissues with high metabolic rates such as liver, heart, skeletal muscle, and kidney and in steroidogenic organs such as the adrenals [12]. PPARγ has three isoforms (PPARγ1, γ2, and γ3) and is expressed in brown and white adipose tissue, large intestine, to a lesser extent in immune cells (monocytes, macrophages, Peyer's patches of the digestive tract), the mucosa of colon and cecum, and placental trophoblasts [13–16]. PPARβ/δ is distributed in all tissues tested with particularly high expression in placenta and large intestine [8, 17, 18]. PPARα and PPARγ are involved in adipocyte differentiation, lipid metabolism, insulin action, and in the regulation of inflammatory responses [1, 5, 16], particularly involving the macrophage [19]. PPARβ/δ is known to be involved in lipid metabolism and inflammation, as well as keratinocyte differentiation and wound healing [5, 20, 21].

The PPAR system is intimately involved in cardiovascular disease, obesity, as well as pregnancy-specific diseases [6, 22]. Over the past decade studies have shown that all three PPAR isotypes are expressed in human placental trophoblast cells [11] and that they are involved in the regulation of pregnancy physiology and its clinical complications. Physiological and

Table 1: Effects of physiological and pathophysiological conditions on PPAR.

	Influence on PPAR action		
Conditions	PPAR-action	Model	Reference
Diabetes	Increases PPARγ in skeletal muscle	Murine	Park et al. [22]
Age	Increases PPARγ in subcutaneous fat in older man	Human	Imbeault et al. [23]
	Decreases PPARα in heart	Murine	Iemitsu et al. [24]
Hypertension	Increases PPARα and γ in aorta and mesenteric arteries	Murine	Diep and Schiffrin [25]
Diet	Soy extract increases PPARα and γ in macrophages	In vitro	Mezei et al. [28]
	High-fat diet increases adipose tissue expression of PPARγ and induces PPARγ2 mRNA expression in liver (obese mice)	Murine	Vidal-Puig et al. [26]
	Hyperlipid diet reduces PPARγ in colonic epithelium	Murine	Delage et al. [29]
	Low-calorie diet decreases PPARγ in subcutaneous fat	Human	Bastard et al. [27]
Exercise	Increases PPARγ DNA binding activity in fat depots	Murine	Petridou et al. [30]
	Increases PPARα in heart	Murine	Iemitsu et al. [24]
	Increases PPARβ/δ in skeletal muscle	Human	Fritz et al. [34]
Obesity	Increases of PPARγ2 and PPARγ2/PPARγ1 ratio in adipose tissue	Human	Vidal-Puig et al. [31]
Metabolic syndrome	Dominant-negative mutation in PPARγ induces metabolic syndrome	Human	Savage et al. [35]
Insulin resistance (IR)	Pioglitazone ameliorates IR	Murine	Ding et al. [33]
	PPARγ Ala allele protects against hyperinsulinemia	Human	Jaziri et al. [32]
Vitamin A	Increases PPARγ in colonic mucosa	Murine	Delage et al. [29]

Table 2: Effects of metabolic conditions on pregnancy-specific diseases (GDM: gestational diabetes mellitus; PE: preeclampsia; IUGR: Intrauterine growth restriction; −: reduced risk; +: increased risk).

	Influence on pregnancy-specific diseases			
Conditions	GDM	PE	IUGR	Reference
Diabetes	—	+	—	Ostlund et al. [36]
Advanced maternal age	+	+	+	Delbaere et al. [53] Odibo et al. [37]
Hypertension	—	+	+	Sibai et al. [38]
Optimal nutrition	−	−	−	Artal et al. [41] Saftlas et al. [43] Scholl et al. [39]
Optimal exercise	−	−	—	Artal et al. [41] Zhang et al. [44] Sorensen et al. [42] Saftlas et al. [43]
Obesity	+	+	+	Cedergren [48] Saftlas et al. [43] O'Brien et al. [47] Ros et al. [45] Sebire et al. [46] Bodnar et al. [49]
Metabolic syndrome	+	+	+	Ray et al. [50]
Insulin resistance	—	+	—	Wolf et al. [51]
Periconceptional multivitamins	—	−	—	Bodnar et al. [52]

pathophysiological conditions that modulate the PPAR system [22–35] influence the risk and course of preeclampsia (PE), gestational diabetes mellitus (GDM), or intrauterine growth restriction (IUGR) [36–53]. Some of these diseases and factors involving the PPAR system are summarized in Tables 1 and 2.

In early pregnancy, immediately after embryonic implantation, major maternal physiologic changes occur in the cardiovascular, hepatic, and endocrine systems with resultant anatomical and metabolic modifications that serve to promote maternal immune tolerance of the conceptus and to provide the fetus with its increased nutritional needs [54, 55]. Metabolic changes (including increased availability of glucose, low density lipoprotein, and fatty acids) increased insulin resistance and altered amino acid metabolism, immunologic, and hematologic changes (including an increase in plasma volume). Establishment of a thrombophilic state and extensive placental and decidual angiogenesis are observed in pregnancy, and these changes require a complex activation of regulating mediators [56–58].

Pregnancy complications result when the mother and/or fetus fail to adapt to these new metabolic, angiogenic, and thrombogenic challenges. Women with preexisting compromise to their vascular homeostasis, such as underlying

Table 3: PPAR knock out models and placental pathology (PRIP: peroxisome proliferator-activated receptor-(PPAR) interacting protein; RAP 250: nuclear receptor-activating protein 250).

PPAR knockout model	Placental pathology	Lethality	Reference
PPARα	No significant effect on placentation	20%	Yessoufou et al. [76]
PPARβ/δ	Poor placentation	>90%	Barak et al. [77]
PPARγ	Poorly developed labyrinth	100%	Barak et al. [15] Kubota et al. [82]
PPARγ coactivator PRIP	Reduced spongiotrophoblast layer	100%	Zhu et al. [79]
PPARγ coactivator RAP250	Reduced spongiotrophoblast layer	100%	Antonson et al. [80]
RXRα or β	Lack of labyrinth zone	100%	Sapin et al. [81]

hypertension, diabetes mellitus, or metabolic syndrome, have a significantly increased risk of developing pregnancy complications (see Table 2). Placenta-associated complications also can lead to impaired growth or fetal demise [59, 60]. These placental conditions share vasculopathological mechanisms in common with atherosclerosis and represent early markers for maternal risk of cardiovascular disease [61, 62] and hypertension [61, 63, 64]. Curiously, a prior history of preeclampsia appears to confer protection against the future development of endometriosis and some cancers [65, 66].

PPARs can be activated by natural ligands, like prostaglandins (PGs), fatty acids, and their derivatives, as well as by synthetic ligands. PPAR medications have been developedand discovered to be relatively safe drugs with benefits in multiple disease states including diabetes and cardiovascular disease [67]. Fibrate drugs used to treat hyperlipidemia, and thiazolidinedione drugs used to treat type 2 diabetes are potent and relatively specific ligand activators of PPARα and γ, respectively, and are widely used clinically [68, 69]. A number of naturally-occurring PPAR ligands have been identified, including long-chain fatty acids (C16 and greater), eicosanoids such as 8(S)-HETE (PPARα) and 9- and13-HODE (PPARγ), and PGs such as PGA_1, which binds to PPARα, PPARβ/δ, and 15-deoxy-delta^{12,14}-prostaglandin J_2(15dPGJ_2), which in turn binds to PPARγ [70–72]. Both the expression of PPAR and the production of their potential ligands are altered during pregnancy and its related diseases. We postulate that pathologic diversion of fatty-acid metabolism away from the production of eicosanoid ligands in preeclampsia and gestational diabetes might be corrected using synthetic ligands.

2. PPARs IN TROPHOBLAST INVASION AND PLACENTAL DEVELOPMENT

In first trimester, human placental bed biopsies, PPAR-γ is expressed predominantly in invasive trophoblasts, whereas in the second-trimester PPARγ is expressed in the columns of anchoring villi and cytotrophoblasts [73, 74]. In the third trimester, PPARγ principally localizes to extravillous cytotrophoblasts (EVCT) and villous syncytiotrophoblasts [75], where it appears to regulate placental hormone production and secretion. Although the focus of this review is to summarize findings on PPAR/RXR heterodimers in human placentation, much of the direct evidence for a role of these receptors in trophoblast invasion and placental development has emerged from studies in knockout mouse models. This topic is reviewed comprehensively in Schaiff et al. [3], and is summarized briefly here and in Table 3 [76–81].

PPARγ/RXRα heterodimers play a key regulatory role in murine placental development. PPARγ deficiency was shown to interfere with terminal trophoblast differentiation and placental vascularization [78]; embryos without this gene show massive placental defects that can be rescued by restoration of the trophoblast PPARγ gene via tetraploid chimeras [15]. Deletion of RXRα and RXRβ also leads to embryo lethality [15, 81, 83]. Both PPAR-interacting protein (PRIP) and nuclear receptor-activating protein 250 (RAP250) encode nuclear receptor coactivators that associate with PPARs, RXRs, and other nuclear receptor proteins. Genetic disruption of PRIP or RAP250 in mouse models results in embryonic lethality at postconception days 11.5 and 13.5, respectively [79, 80]. Placentas of PRIP (−/−) and RAP250 (−/−) embryos exhibited dramatically reduced spongiotrophoblast and labyrinth layers as well as failure of blood vessel maturation in the region bordering the spongiotrophoblast [79, 80].

In addition to placentation per se, PPARγ appears to play an important role in the uterine preparation for embryonic implantation. Peeters et al. demonstrated that PPARγ ligands reduced the production of the endometrial angiogenic factor VEGF, and postulated that this pathway might influence early embryonic vascularization [84]. By contrast, PPARγ agonists induce angiogenesis in cardiac myofibroblasts, smooth muscle cells, and macrophages [85–87]. Recent preliminary data by our lab and others suggest that the PPARγ system also stimulates VEGF expression in trophoblast (JEG-3) cells (Depoix et al., unpublished).

The functional role of PPARγ activity is well studied in trophoblast physiology (Table 4). PPARγ agonists inhibit invasion of cultured EVCT isolated from human first-trimester placenta, whereas PPARγ antagonists promoted EVCT invasion and repressed the PPARγ agonist-mediated effects [78]. PPARγ controls mucin (MUC)-1 transcription and regulates maternal-fetal transport in mouse models [88]. Moreover, PPARγ and RXRα play a role in human chorionic gonadotropin (hCG) expression, trophoblast differentiation, and regulation of fatty acid transport and storage in human placental trophoblasts [89, 90]. PPARγ diminishes leptin-induced inflammatory responses in the human placenta [91] and inhibits PAPP-A expression [92].

Table 4: PPAR action in trophoblast development and placental function (MUC-1: mucin-1; EVCT: extravillous cytotrophoblast; hCG: human chorionic gonadotropin; Th2 T-helper 2 cell).

PPAR action in trophoblast development and placentation			
PPAR	PPAR action	Model	Reference
PPARγ	Inhibits EVCT invasion	In vitro	Fournier et al. [78]
	Promotes trophoblast differentiation hCG secretion	In vitro	Tarrade et al. [89]
	Induces hCG production	In vitro	Schild et al. [93]
	Antiinflammatory	In vitro	Lappas et al. [91]
	Regulates fatty acid transport	In vitro	Schaiff et al. [90]
	Increases VEGF expression	In vitro	Depoix, unpublished
	Terminal differentiation, placental vascularization	Murine	Barak et al. [15]
	Controls MUC-1 expression	Murine	Shalom-Barak et al. [88]
	Stimulates trophoblast maturation	Murine	Asami-Miyagishi et al. [94]
	Modulates placental lipid metabolism	Murine	Capobianco et al. [95]
PPARβ/δ	Promotes placental development	Murine	Nadra et al. [8]
PPARα	Regulates placental lipid transfer	Murine/Human	Wang et al. [74]
	PPAR action in pregnancy		
PPARγ	Antiinflammatory	In vitro	Lappas et al. [96]
	Involved in inflammatory control and remodeling in the placenta	In vitro	Marvin et al. [97]
	Increased circulating PPARγ activators in normal pregnancy	In vitro/human	Waite et al. [73]
	Decreases in fetal membrane with labor	Human	Dunn-Albanese et al. [98]
PPARβ/δ	Increases in amnion with labor	Human	Berry et al. [99]
PPARα	Stimulates Th2 cytokine pattern during pregnancy	Murine	Yessoufou et al. [76]
	Declines in choriodecidua with labor	Human	Berry et al. [99]

Regulation of PPARγ in human placental tissues is thought to occur through natural ligands (e.g., 15dPGJ2, 9-HODE, 13-HODE, and 15-HETE) through direct binding to the receptor's ligand binding pocket [11, 100]. These ligands are likely to be synthesized locally within the placenta. Furthermore, crosstalk between the mitogen-activated protein kinase (MAPK) p38 and PPARγ occurs within cultured trophoblast cells [101]. PPARγ decreases IGFII secretion and is thought to inhibit trophoblast invasion via the PAPP-A cascade [92].

In young PPARα knock out mice, no major phenotypic differences of gross pathology of internal organs were described [76, 102]. However, disturbance of the Th1/Th2 T-lymphocyte ratio, rather than placental malformation, is thought to be responsible for an increased abortion rate (20%) in PPARα null mice. During normal pregnancy Th1 cytokines are downregulated and Th2 cytokines are upregulated [103].

The third distinct PPAR, PPARβ/δ also is essential for placentation as demonstrated in PPARβ/δ knockout mice (Table 3) [77], and is involved in the regulation of implantation in other animal models [17, 104, 105]. The implantation of cultured embryos is enhanced by PPARβ/δ activation and this receptor even has been postulated as a novel therapeutic target to improve clinical IVF outcomes [104]. PPARβ/δ is induced during decidualization of the implantation site and requires close contact with the blastocyst. PPARβ/δ null mice die between 9.5 to 10.5 embryonic days due to abnormal cell-cell communication at the placental-decidual interface [8].

Together these data suggest that PPARs are required not only for trophoblast invasion and differentiation but also for establishment of the placental maternal-fetal transport.

3. PPARS AND PREGNANCY

Based on its regulatory functions and known eicosanoid ligands, PPARγ has emerged as an excellent candidate to play a role in the regulation of maternal metabolism, maintenance of uterine quiescence, and onset of labor by regulating proinflammatory cytokines and prostaglandins (Table 4). Normal pregnancy is accompanied by changes in lipid and glucose metabolism, but further dysregulation of these pathways can lead to pregnancy complications such as PE or GDM. Hence, PPAR regulators of these metabolic pathways might be expected to be important in human pregnancy.

Some of our initial studies in this field were designed to screen for potential activators of PPARγ in the circulation of pregnant women. Human choriocarcinoma JEG-3 cells were transfected with peroxisome-proliferator responsive reporter plasmids; and pooled sera from pregnant and nonpregnant women were added to the cell culture medium [73]. Peroxisome proliferator responsive element (PPRE) luciferase reporter activation was dramatically increased by sera from pregnant women compared to nonpregnant women (Figures 1 and 2). We showed that PPARγ (and to some extent PPARα) activity is increased from the earliest stages of pregnancy (Figure 2). The findings suggested that circulating PPARγ-activating factors, presumably eicosanoids, were

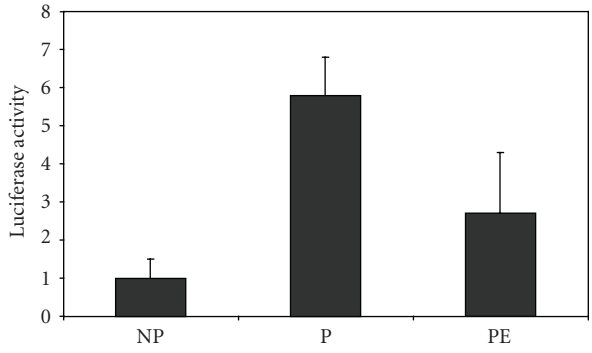


Figure 1: JEG-3 cells were transfected with PPRE-luciferase reporter vectors and treated with pooled sera (10%) from nonpregnant (NP), pregnant (P) and preeclamptic (PE) women. Luciferase acitivity, relative to cells treated with 10% dextran charcoal-shipped fetal calf serum (DCSS), is reported on the ordinate.

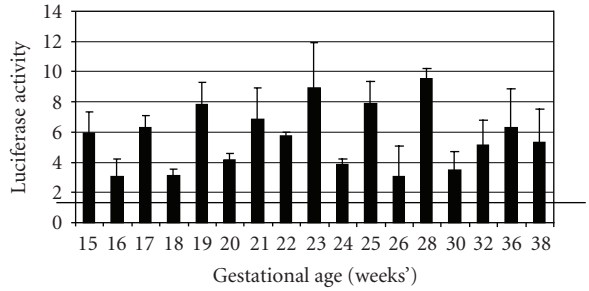


Figure 2: PPARγ activation is present throughout the course of normal pregnancy. All serum samples were collected from the same subject and PPRE-luciferase reporter experiments were performed using 10% serum as described in Figure 1. Luciferase activity was normalized to DCSS to determine relative activation. Black horizontal bar represents the level of signaling seen with 10% serum from the same woman six weeks after delivery.

present throughout the course of gestation. We hypothesized that activation of PPARγ by sera of pregnant women is a regulatory adaptation of the maternal organism to increased lipid and glucose loading in pregnancy [73].

It also has been hypothesized that PPARγ activation regulates uterine quiescence by influencing Nuclear Factor-Kappa B (NFκB) and cyclooxygenase (COX-2) expression [96, 97, 106]. Reciprocal expression of PPARγ and (COX)-2 in human term placenta suggests a role of the PPAR system in the initiation of labor [98]. Under conditions of high PPARγ expression, antiinflammatory actions dominate; however, with onset of labor PPARγ levels drop and COX-2 concomitantly increases in the fetal membranes [98]. Elevated COX-2 activity in the human amnion is observed in the settings of term and idiopathic preterm labor, contributing to the generation of uterotonic prostaglandins (PGs), which are known to participate in parturition [107]. PPARγ ligands have been shown to antagonize NF-κB activation and reduce inflammatory cytokine gene expression (IL-1β, IL-6, IL-10 and TNF-α) and COX-2 [108]. Both natural (e.g., 15dPGJ2) and synthetic ligands (e.g., troglitazone) were shown to have anti-inflammatory effects in human gestational tissues, significantly decreasing basal and LPS-stimulated PGE_2 and $PGF_{2\alpha}$ release from placenta and amnion [108]. $PGF_{2\alpha}$, also a marker of oxidative stress, is increased in women with preeclampsia [109]. Given the inflammatory changes observed in pregnancy-specific diseases, a potential role of PPAR agonist treatment has been entertained for the treatment of PE, GDM, and other pregnancy-specific diseases such as the prevention of preterm labor [96].

PPARα and β/δ also play a role in maintaining pregnancy and parturition. PPARα and β/δ are expressed in the amnion, choriodecidua, and villous placental tissues. Data from PPARα knockout mice suggest that PPARα maintains pregnancy by stimulating a Th2 cytokine response [76]. In normal pregnancy, expression of PPARα declines in the choriodecidua with the onset of labor [99]. By contrast, PPARβ/δ expression, which is temporally upregulated between the first and third trimester of pregnancy [99], increases further in the amnion coincidental with the onset of labor [99].

Few studies have elucidated substantial risk of PPAR agonists during pregnancy in animal models, but these drugs carry a "C" classification from the FDA. For example, rosiglitazone did not damage blastocyst development in vitro or harm mouse fetuses when given during murine pregnancy [110]. While the use of rosiglitazone during pregnancy is generally considered to be safe [110]; more data need to be acquired before these drugs can be recommended.

4. PPARs AND PREGNANCY-SPECIFIC DISEASES

Failure of metabolic adaptation to pregnancy can result in pregnancy-specific complications such as PE and GDM. We and others have postulated that angiogenic factors and cytokines that lead to pathological gestational changes are likely to be regulated by the PPAR system (Table 5).

4.1. PPARs and preeclampsia

PE is a multifactorial, pregnancy-related disorder that is defined by new-onset hypertension and proteinuria after 20 weeks of gestation [117]. PE is a common cause of maternal and infant morbidity and mortality worldwide, and is responsible for about 20% of pregnancy-related maternal deaths in the US [118]. Women with PE have increased insulin resistance as well as hypertriglyceridemia relative to normal pregnant women [119]. To date, no effective treatment has been found that either prevents or reverses the development of the disease. Modern concepts of PE pathophysiology invoke a two-stage process. The first stage is believed to be initiated by impaired trophoblast invasion and abnormal uterine vessel remodeling. The second stage is postulated to result from circulating factors claimed to be derived from the ischemic placenta that stimulate an inflammatory activation of maternal vascular endothelial cells. PE presents clinically in the second or third trimester, however, fundamental inflammatory and angiogenic biomarkers in the serum are detectable as early as the first trimester in women with PE. Elevated concentrations of IL-2, TNFα, and sVEGFR-1

Table 5: PPAR in pregnancy-specific diseases.

PPAR	PPAR-action	Disease	Model	Reference
PPARγ	Reduced circulating PPARγ activators in serum from women with PE	PE	In vitro	Waite et al. [111]
	Placental 15dPGJ$_2$level are decreased in diabetes	GDM	Murine	Capobianco et al. [95]
	Association of PPAR-γ2 Pro12Ala with weight gain	GDM	Human	Tok et al. [112]
	Placental 15dPGJ$_2$levels are decreased	GDM	Human	Javerbaum et al. [113]
	Decreased	Hydatidiform mole	Human	Capparuccia et al. [114]
	Decreased	Choriocarcinoma	Human	Capparuccia et al. [114]
	Placental PPAR expression is not involved	IUGR	Human	Rodie et al. [115]
	Association of PPAR-γ2 Pro12Ala polymorphism	Preterm birth	Human	Meirhaeghe et al. [116]
PPARα	Lack of PPAR-α upregulates Th1 cytokines	Abortion/neonatal mortality	Murine	Yessoufou et al. [76]

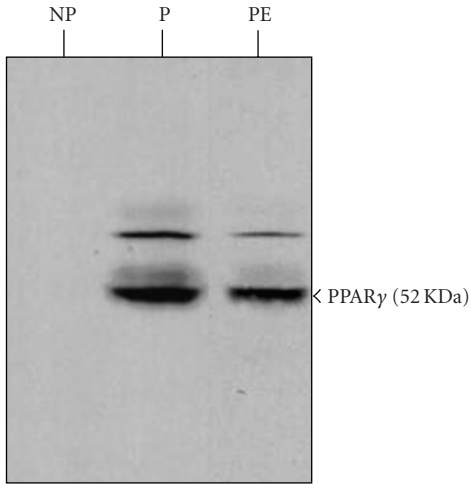


Figure 3: Immunoblot of JEG-3 cells treated with pooled sera (10%) from nonpregnant (NP), pregnant (P), and preeclamptic (PE) women. Cell lysates were analyzed using a specific mouse anti-human PPARγ monoclonal antibody. Equal amounts of protein (50 μg) were loaded into each lane. Factors in pregnant serum upregulate JEG-3 PPARγ expression. A decrease in PPARγ protein was observed in cells exposed to PE sera (PE) compared to sera from normal pregnant women (P).

and reduced concentrations of PlGF, IGFBP-1, and HLA-G in the maternal serum precede the clinical manifestations of PE [119–123].

While the cause of PE remains unknown, several environmental and genetic risk factors have been identified (Table 2). Relevant to this review are hypertension, diabetes, and high (>29) body mass index (BMI) [47, 124, 125]. Black race also appears to be a risk factor for PE [126] although this may be confounded by increased rates of the above risk factors. Key inflammatory and angiogenic pathways involved in the pathogenesis of PE are regulated by the PPAR system, which itself is influenced by environmental and genetic factors. We believe that exogenous and endogenous lipid regulators of PPAR play a role in maternal metabolism and immune functionin normal and pathological pregnancies. For example, dietary factors and physical activity that modulate the PPAR system have been shown to reduce the risk and course of PE (Table 2).

Similarly, genetic variations in the PPARγ gene have been proposed to modify the risk of PE. For example, the Pro467Leu mutation of PPARγ [127–129] is a dominant negative mutant resulting from a C-to-T transition in exon 6. A report of two individuals (one woman, one man) with this mutation showed that they developed type 2 diabetes at young ages (26 and 27 years at diagnosis), as well as early hypertension (37 and 27 years at diagnosis). Intriguingly, the woman had two pregnancies, both of which were complicated by severe PE. The Pro12Ala polymorphism occurs in PPARγ2 [130], a second isoform of PPARγ that is expressed mainly in adipose tissue. This mutation is the result of a C-to-G transversion in exon B. This is by far the most studied allelic variation in any PPAR, and occurs at a rate of about 12% in the Caucasian US population. While the resulting phenotype is highly diverse and even apparently contradictory, it appears that the penetrance of this mutation is influenced by other genetic, environmental, ethnic, and gender differences. The studies generally agree that the presence of the Ala allele is associated with increased BMI, an independent risk factor for PE. Thus, this polymorphism is a candidate affecting pregnancy outcome. Preliminary data of a study on the PPAR gene variations (in PPAR gene) showed no association with PE or severity of PE in a Finnish population [131]. Further studies on the association of PPAR α, β, and γ gene variations of mothers and offspring and pregnancy-specific diseases need to be performed in different ethnic populations.

PE is marked by hyperlipidemia, and is characterized by a state of oxidative stress. Circulating lipids in PE women are more highly oxidized, and oxidized low-density lipoproteins (oxLDLs), in particular, are highly elevated [132]. Given the circulating plasma lipid disturbances in PE, our group performed experiments comparing sera from normal and PE patients. We found that serum from women with severe PE had reduced levels of PPAR activating lipids compared with serum of parity and gestational age-matched women and also diminished the expression of PPARγ in trophoblast cells (Figures 1 and 3) [111]. The reduction of transcriptional

activity observed in preeclamptic women's sera was shown for PPARγ and PPARα, however not for PPARβ/δ or RXR. The reduction in potential circulating PPAR activatorswas observed weeks and sometimes months before the onset of maternal symptoms and clinicaldiagnosis of PE [133]. Our results are consistent with other clinical evidence that antiinflammatory regulation is challenged and further compromised in the maternal syndrome of PE. Normal pregnancy manifests as a physiologic inflammatory state postulated to be tolerated to serve the nutritional needs of the fetus, whereas, in PE regulatory inflammatory mechanisms are excessively amplified, leading to vascular damage in the mother [133]. In this "hyperinflammatory" state of PE [134], the cytokines TNFα and IL-1β which are typically controlled by the NF-κB pathway in a negative-feedback loop with PPAR, are elevated [26, 60, 119]. Elevated inflammatory parameters in PE accompany altered levels of PG metabolites and circulating fatty acids. As noted, PG metabolites as well as fatty acids are important ligands of the PPAR system [135]. PG metabolism is altered during normal pregnancy with levels of vasorelaxants suchas prostacyclin increasing, whereas vasoconstrictive prostaglandin levelstend to be suppressed [136]. Failure of these alterations have been suggested to lead to pregnancy complications (e.g., PE) [137]. For example, $PGF_{2\alpha}$, which itself is stimulated by factorsin the plasma of women with PE [138], can inhibit PPARγ effects [135]. Levelsof circulating free fatty acids are in the normal range duringmost of pregnancy, but rise dramatically during the final weeks of pregnancy and drop precipitously at term [136]. In PE these levels are increased from 20 weeks' gestation [133, 139]. We postulate that altered PG metabolism in this setting [138] results in decreased PPARγ ligation and subsequent cytokine activation. If this proposal is supported by more data, the use of PPAR ligands might be proposed to ameliorate symptoms such as hypertension and inflammation. Unfortunately, at present, the mechanism and site of this salutary of PPAR ligand effect remain unknown in pregnancy, confounded by PPAR expression in many cell types, including endothelial cells.

4.2. PPARs and gestational diabetes

During normal pregnancy, maternal lipid, and glucose metabolism is profoundly altered [140]. The developing fetus uses glucose as its predominant energy source, which puts a continuous demand on the mother to provide this substrate [141]. This constant need for glucose results in frequent hypoglycemia and postprandial hyperglycemia during normal pregnancy [141]. Problems with energy metabolism such as GDM are not uncommon and are often observed in susceptible women at this time. GDM is defined as any degreeof glucose intolerance with onset or first recognition during pregnancy. In women with GDM, defective β-cells function cannot adequately compensate for free fatty acid-mediated insulin resistance [142]. As elsewhere in our society, the incidence of obesity, diabetes, and gestational diabetes mellitus are increasing in the pregnant population [143]. In the United States, the incidence of obesity among pregnant women ranges from 18.5% to 38.3% [144]; obesity comprises a major risk factor for GDM [145]. Morphological changes have been identified in the syncytiotrophoblast, cytotrophoblast, trophoblastic basement membrane, and fetal vessels within the placentae of these cases [146]. GDM is associated with several severe neonatal complications (such as macrosomia, brachial plexus palsy, premature delivery, IUGR, and intrauterine death) and maternal birth injuries also are common [125, 147]. Furthermore, GDM has emerged as a risk factor for the development of diabetes mellitus type 2 (DM2) and cardiovascular disease in later life and shares a number of epidemiologic, pathophysiologic, and genetic characteristics with DM2 [148]. GDM also has detrimental effects on the postnatal infants [149].

The PPAR system regulates the metabolic and pathways involved in the establishment of GDM. PPAR-agonists have antidiabetogenic, antiinflammatory, and antioxidant effects, which are all potentially beneficial in the treatment of GDM [5].

Environmental factors, such as diet and exercise and genetic factors influence PPARα, γ activity [130, 150] as well as the risk for insulin resistance and GDM (Table 2). Exercise activity initiated prepregnancy was shown to reduce the risk of GDM and its complications [40, 41, 44, 151, 152]. Nutritional counseling, moderate physical exercise, weight loss, and diet are successful therapies in some women with GDM, improving glycemic control, reducing the incidence of LGA infants, and decreasing the need for cesarean deliveries for cephalopelvic disproportion [41, 153].

Candidate genes for GDM risk include TNFα, β3 adrenoreceptor (ADRB3), and PPARα and γ. The PPARγ Pro12Ala polymorphism was not associated with increased insulin resistance in Turkish women with GDM, however it was associated with weight gain [112]. The PPARγ coactivator-1alpha (PGC-1) polymorphism also failed to be associated with the development of GDM [154]. More studies on the association of various genetic PPARα and γ variants and GDM in different ethnic populations will be of interest.

$15dPGJ_2$ is a potent antiinflammatory agent that represses the expression of a number of inflammatory genes and regulating factors including the transcription factor NF-κB [33, 108]. The concentration of $15dPGJ_2$ was reduced in placentae from diabetic rats (Table 5) [95]. Placental $15dPGJ_2$ was noted to be diminished in women with gestational and pregestational diabetes when compared to controls, whereas levels of nitric oxide (a stimulator of placental invasiveness, differentiation, and proliferation) were higher in term placental explants from diabetic patients when compared to controls [113]. As PPARγ can prevent nitric oxide overproduction in placenta from pregestational diabetic women [113], it may have the potential to improve fetal outcome in this condition.

Sulfonylurea agents including gliumepiride and glibenclamide exhibit PPARγ activity [155]. A randomized controlled trial to test the effectiveness and safety of the sulfonylurea agent glyburide in the management of women with GDM showed similar efficacy to insulin treatment [156]. Both the insulin- and glyburide-treated women were able to

achieve satisfactory glucose control and had similar perinatal outcome [156].

4.3. PPARs and other pregnancy-specific diseases

Trophoblast research has emphasized the similarities between the proliferative, migratory, andinvasive properties of placental cells and those of cancer cells [157]. PPARγ, PPARβ/δ, and RXR appear to be linked to gestational trophoblastic neoplasms, conditions associated with malignant trophoblast behavior [114]. PPARγ agonists inhibit invasion of normal extravillous cytotrophoblast isolated from human first-trimester placenta, and PPAR activity has been shown to be downregulated in trophoblastic diseases including hydatidiform mole and choriocarcinoma [114].

PPARγ has an effect on fetal and placental size influencing intrauterine growth. In an intrauterine growth restriction (IUGR) model, glucocorticoids inhibited fetal and placental growth partly by suppression of PPARγ in the labyrinth zone of the placenta [158]. Activation of PPARγ in the labyrinth trophoblasts is hypothesized to induce angiogenic factors and stimulate the growth of fetal blood vessels, thereby promoting placental growth. However, treatment of pregnant mice with rosiglitazone led to reduced thickness of the spongiotrophoblast layer and the surface area of labyrinthine vasculature, and it altered expression of proteins implicated in placental development [159].

In vitro and in vivo experiments as well as animal models studies suggest a link between the PPAR system and gestational duration, preterm labor, and birth weight [116]. Variations in the PPAR genes influence other pregnancy-related mechanisms including birth weight and gestational duration. In an Irish population, the PPARγ Ala12 allele was associated with shorter gestational duration [116].

PPAR ligands regulate apoptotic mechanisms involved in rupture of the fetal membranes and may play a role in preterm delivery, a condition associated with increased risk of neonatal sepsis and newborn trauma [160]. 15d-PGJ_2induced morphological characteristics of apoptosis within 2 hours in an amniotic cell line [160]. In addition, ciglitizone also induced apoptosis, whereas rosiglitazone had no effect on cell viability [160]. Prevention of apoptosis may have therapeutic potential in preterm labor and premature rupture of the membranes and necessitates further investigations.

Interestingly, PPARα deficiency is associated with miscarriage, neonatal mortality, and a shift from Th2 to a Th1 cytokine phenotype [76]. Th1 predominant immunity is closely associated with inflammation, endothelial dysfunction, and pregnancy complications. For example, interferonγ is significantly reduced in the spleens of PPARα null mice [76]. Twenty percent of PPARα knockout mice aborted, and offspring of PPAR-α null mice exhibited increased neonatal mortality (13.3%). However the mechanism whereby PPARα induces a Th2 phenotype shift remains to be determined. PPARγ ligands also were shown to decrease production of inflammatory ligands in activated macrophages and T cells and to induce a shift from Th1 to Th2 cytokine phenotype [161, 162].

5. CONCLUSIONS

PPARs are involved in trophoblast invasion, placental development, parturition, and pregnancy-specific diseases, particularly PE and GDM. The role of the PPAR system in pregnancy under physiologic and pathologic conditions has remained partly unclear due to lack of knowledge about endogenous PPAR ligands. Pharmacological ligand research is ahead of the identification of physiologic ligands. Partially characterized inflammatory, angiogenic, and metabolic disturbances in pregnancy-related diseases suggest that these synthetic PPAR agonists may be of potential use in these conditions. Ongoing basic studies have elucidated the metabolic, antiinflammatory, and angiogenic benefits of PPAR$\alpha/\beta/\delta$ and PPAR$\gamma/\beta/\delta$ dual agonists and PPAR pan agonists for treatment purposes. However, some experimental and clinical data have uncovered unfortunate side effects of PPAR ligands, including cancer progression and increased cardiac event rates. New generations of PPAR modulators are under development and these promise to be more receptor-specific, and hopefully will activate only a specific subset of target genes and metabolic pathways to reduce untoward side effects. The potential role of PPARs in regulation of inflammation and angiogenesis is intriguing and warrants further studies. We submit that PPAR agonists may become beneficial drugs for pregnancy-specific diseases, once their risks have been fully evaluated.

ACKNOWLEDGMENT

The authors' studies described in this article were supported by NIH Grants P01-HD30367 and R01-HL73469.

REFERENCES

[1] G. Rizzo and S. Fiorucci, "PPARs and other nuclear receptors in inflammation," *Current Opinion in Pharmacology*, vol. 6, no. 4, pp. 421–427, 2006.

[2] A. Margeli, G. Kouraklis, and S. Theocharis, "Peroxisome proliferator activated receptor-γ (PPAR-γ) ligands and angiogenesis," *Angiogenesis*, vol. 6, no. 3, pp. 165–169, 2003.

[3] W. T. Schaiff, Y. Barak, and Y. Sadovsky, "The pleiotropic function of PPARγ in the placenta," *Molecular and Cellular Endocrinology*, vol. 249, no. 1-2, pp. 10–15, 2006.

[4] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[5] J. Berger and D. E. Moller, "The mechanisms of action of PPARs," *Annual Review of Medicine*, vol. 53, pp. 409–435, 2002.

[6] S. Kersten, B. Desvergne, and W. Wahli, "Roles of PPARs in health and disease," *Nature*, vol. 405, no. 6785, pp. 421–424, 2000.

[7] M. J. Holness, G. K. Greenwood, N. D. Smith, and M. C. Sugden, "Peroxisome proliferator-activated receptor-α and glucocorticoids interactively regulate insulin secretion during pregnancy," *Diabetes*, vol. 55, no. 12, pp. 3501–3508, 2006.

[8] K. Nadra, S. I. Anghel, E. Joye, et al., "Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor β/δ,"

Molecular and Cellular Biology, vol. 26, no. 8, pp. 3266–3281, 2006.

[9] G. Krey, A. Mahfoudi, and W. Wahli, "Functional interactions of peroxisome proliferator-activated receptor, retinoid-X receptor, and Sp1 in the transcriptional regulation of the acyl- coenzyme-A oxidase promoter," *Molecular Endocrinology*, vol. 9, no. 2, pp. 219–231, 1995.

[10] Y. Guan, Y. Zhang, L. Davis, and M. D. Breyer, "Expression of peroxisome proliferator-activated receptors in urinary tract of rabbits and humans," *American Journal of Physiology - Renal Physiology*, vol. 273, no. 6, pp. F1013–F1022, 1997.

[11] T. Fournier, V. Tsatsaris, K. Handschuh, and D. Evain-Brion, "PPARs and the placenta," *Placenta*, vol. 28, no. 2-3, pp. 65–76, 2007.

[12] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[13] L. Fajas, J.-C. Fruchart, and J. Auwerx, "PPARγ3 mRNA: a distinct PPARγ mRNA subtype transcribed from an independent promoter," *FEBS Letters*, vol. 438, no. 1-2, pp. 55–60, 1998.

[14] P. Peraldi, M. Xu, and B. M. Spiegelman, "Thiazolidinediones block tumor necrosis factor-α-induced inhibition of insulin signaling," *Journal of Clinical Investigation*, vol. 100, no. 7, pp. 1863–1869, 1997.

[15] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[16] X. Zhang and H. A. Young, "PPAR and immune system—what do we know?" *International Immunopharmacology*, vol. 2, no. 8, pp. 1029–1044, 2002.

[17] E. Lord, B. D. Murphy, J. A. Desmarais, S. Ledoux, D. Beaudry, and M.-F. Palin, "Modulation of peroxisome proliferator-activated receptor δ and γ transcripts in swine endometrial tissue during early gestation," *Reproduction*, vol. 131, no. 5, pp. 929–942, 2006.

[18] H. Higashiyama, A. N. Billin, Y. Okamoto, M. Kinoshita, and S. Asano, "Expression profiling of Peroxisome proliferator-activated receptor-δ (PPAR-δ) in mouse tissues using tissue microarray," *Histochemistry and Cell Biology*, vol. 127, no. 5, pp. 485–494, 2007.

[19] A. Chawla, Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans, "PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation," *Nature Medicine*, vol. 7, no. 1, pp. 48–52, 2001.

[20] D. K. Krämer, L. Al-Khalili, B. Guigas, Y. Leng, P. M. Garcia-Roves, and A. Krook, "Role of AMP kinase and PPARδ in the regulation of lipid and glucose metabolism in human skeletal muscle," *Journal of Biological Chemistry*, vol. 282, no. 27, pp. 19313–19320, 2007.

[21] M. Adjuik., A. Babiker, P. Garner, P. Olliaro, W. Taylor, and N. White, "Artesunate combinations for treatment of malaria: meta-analysis," *The Lancet*, vol. 363, no. 9402, pp. 9–17, 2004.

[22] K. S. Park, T. P. Ciaraldi, L. Abrams-Carter, S. Mudaliar, S. E. Nikoulina, and R. R. Henry, "PPAR-γ gene expression is elevated in skeletal muscle of obese and type II diabetic subjects," *Diabetes*, vol. 46, no. 7, pp. 1230–1234, 1997.

[23] P. Imbeault, H. Vidal, A. Tremblay, et al., "Age-related differences in messenger ribonucleic acid expression of key proteins involved in adipose cell differentiation and metabolism," *Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 2, pp. 828–833, 2001.

[24] M. Iemitsu, T. Miyauchi, S. Maeda, et al., "Aging-induced decrease in the PPAR-α level in hearts is improved by exercise training," *American Journal of Physiology - Heart and Circulatory Physiology*, vol. 283, no. 5, pp. H1750–H1760, 2002.

[25] Q. N. Diep and E. L. Schiffrin, "Increased expression of peroxisome proliferator-activated receptor-α and -γ in blood vessels of spontaneously hypertensive rats," *Hypertension*, vol. 38, no. 2, pp. 249–254, 2001.

[26] A. Vidal-Puig, M. Jimenez-Liñan, B. B. Lowell, et al., "Regulation of PPAR γ gene expression by nutrition and obesity in rodents," *Journal of Clinical Investigation*, vol. 97, no. 11, pp. 2553–2561, 1996.

[27] J. P. Bastard, B. Hainque, E. Dusserre, et al., "Peroxisome proliferator activated receptor-γ, leptin and tumor necrosis factor-α mRNA expression during very low calorie diet in subcutaneous adipose tissue in obese women," *Diabetes/Metabolism Research and Reviews*, vol. 15, no. 2, pp. 92–98, 1999.

[28] O. Mezei, W. J. Banz, R. W. Steger, M. R. Peluso, T. A. Winters, and N. Shay, "Soy isoflavones exert antidiabetic and hypolipidemic effects through the PPAR pathways in obese zucker rats and murine RAW 264.7 cells," *Journal of Nutrition*, vol. 133, no. 5, pp. 1238–1243, 2003.

[29] B. Delage, C. Bairras, B. Buaud, V. Pallet, and P. Cassand, "A high-fat diet generates alterations in nuclear receptor expression: prevention by vitamin A and links with cyclooxygenase-2 and β-catenin," *International Journal of Cancer*, vol. 116, no. 6, pp. 839–846, 2005.

[30] A. Petridou, S. Tsalouhidou, G. Tsalis, T. Schulz, H. Michna, and V. Mougios, "Long-term exercise increases the DNA binding activity of peroxisome proliferator-activated receptor γ in rat adipose tissue," *Metabolism*, vol. 56, no. 8, pp. 1029–1036, 2007.

[31] A. J. Vidal-Puig, R. V. Considine, M. Jimenez-Liñan, et al., "Peroxisome proliferator-activated receptor gene expression in human tissues. Effects of obesity, weight loss, and regulation by insulin and glucocorticoids," *Journal of Clinical Investigation*, vol. 99, no. 10, pp. 2416–2422, 1997.

[32] R. Jaziri, S. Lobbens, R. Aubert, et al., "The *PPARG* Pro12Ala polymorphism is associated with a decreased risk of developing hyperglycemia over 6 years and combines with the effect of the *APM1* G-11391A single nucleotide polymorphism," *Diabetes*, vol. 55, no. 4, pp. 1157–1162, 2006.

[33] S. Y. Ding, Z. F. Shen, Y. T. Chen, S. J. Sun, Q. Liu, and M. Z. Xie, "Pioglitazone can ameliorate insulin resistance in low-dose streptozotocin and high sucrose-fat diet induced obese rats," *Acta Pharmacologica Sinica*, vol. 26, no. 5, pp. 575–580, 2005.

[34] T. Fritz, D. K. Krämer, H. K. Karlsson, et al., "Low-intensity exercise increases skeletal muscle protein expression of PPARδ and UCP3 in type 2 diabetic patients," *Diabetes/Metabolism Research and Reviews*, vol. 22, no. 6, pp. 492–498, 2006.

[35] D. B. Savage, G. D. Tan, C. L. Acerini, et al., "Human metabolic syndrome resulting from dominant-negative mutations in the nuclear receptor peroxisome proliferator-activated receptor-γ," *Diabetes*, vol. 52, no. 4, pp. 910–917, 2003.

[36] I. Ostlund, B. Haglund, and U. Hanson, "Gestational diabetes and preeclampsia," *European Journal of Obstetrics & Gynecology and Reproductive Biology*, vol. 113, no. 1, pp. 12–16, 2004.

[37] A. O. Odibo, D. Nelson, D. M. Stamilio, H. M. Sehdev, and G. A. Macones, "Advanced maternal age is an independent risk

factor for intrauterine growth restriction," *American Journal of Perinatology*, vol. 23, no. 5, pp. 325–328, 2006.

[38] B. M. Sibai, M. Ewell, R. J. Levine, et al., "Risk factors associated with preeclampsia in healthy nulliparous women. The calcium for preeclampsia prevention (CPEP) study group," *American Journal of Obstetrics & Gynecology*, vol. 177, no. 5, pp. 1003–1010, 1997.

[39] T. O. Scholl, M. Leskiw, X. Chen, M. Sims, and T. P. Stein, "Oxidative stress, diet, and the etiology of preeclampsia," *American Journal of Clinical Nutrition*, vol. 81, no. 6, pp. 1390–1396, 2005.

[40] C. Zhang, M. B. Schulze, C. G. Solomon, and F. B. Hu, "A prospective study of dietary patterns, meat intake and the risk of gestational diabetes mellitus," *Diabetologia*, vol. 49, no. 11, pp. 2604–1613, 2006.

[41] R. Artal, R. B. Catanzaro, J. A. Gavard, D. J. Mostello, and J. C. Friganza, "A lifestyle intervention of weight-gain restriction: diet and exercise in obese women with gestational diabetes mellitus," *Applied Physiology, Nutrition, and Metabolism*, vol. 32, no. 3, pp. 596–601, 2007.

[42] T. K. Sorensen, M. A. Williams, I. M. Lee, E. E. Dashow, M. L. Thompson, and D. A. Luthy, "Recreational physical activity during pregnancy and risk of preeclampsia," *Hypertension*, vol. 41, no. 6, pp. 1273–1280, 2003.

[43] A. F. Saftlas, N. Logsden-Sackett, W. Wang, R. Woolson, and M. B. Bracken, "Work, leisure-time physical activity, and risk of preeclampsia and gestational hypertension," *American Journal of Epidemiology*, vol. 160, no. 8, pp. 758–765, 2004.

[44] C. Zhang, C. G. Solomon, J. E. Manson, and F. B. Hu, "A prospective study of pregravid physical activity and sedentary behaviors in relation to the risk for gestational diabetes mellitus," *Archives of Internal Medicine*, vol. 166, no. 5, pp. 543–548, 2006.

[45] H. S. Ros, S. Cnattingius, and L. Lipworth, "Comparison of risk factors for preeclampsia and gestational hypertension in a population-based cohort study," *American Journal of Epidemiology*, vol. 147, no. 11, pp. 1062–1070, 1998.

[46] N. J. Sebire, M. Jolly, J. P. Harris, et al., "Maternal obesity and pregnancy outcome: a study of 287 213 pregnancies in London," *International Journal of Obesity and Related Metabolic Disorders*, vol. 25, no. 8, pp. 1175–1182, 2001.

[47] T. E. O'Brien, J. G. Ray, and W. S. Chan, "Maternal body mass index and the risk of preeclampsia: a systematic overview," *Epidemiology*, vol. 14, no. 3, pp. 368–374, 2003.

[48] M. I. Cedergren, "Maternal morbid obesity and the risk of adverse pregnancy outcome," *Obstetrics & Gynecology*, vol. 103, no. 2, pp. 219–224, 2004.

[49] L. M. Bodnar, J. M. Catov, M. A. Klebanoff, R. B. Ness, and J. M. Roberts, "Prepregnancy body mass index and the occurrence of severe hypertensive disorders of pregnancy," *Epidemiology*, vol. 18, no. 2, pp. 234–239, 2007.

[50] J. G. Ray, M. J. Vermeulen, M. J. Schull, S. McDonald, and D. A. Redelmeier, "Metabolic syndrome and the risk of placental dysfunction," *Journal of Obstetrics and Gynaecology Canada*, vol. 27, no. 12, pp. 1095–1101, 2005.

[51] M. Wolf, L. Sandler, K. Muñoz, K. Hsu, J. L. Ecker, and R. Thadhani, "First trimester insulin resistance and subsequent preeclampsia: a prospective study," *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 4, pp. 1563–1568, 2002.

[52] L. M. Bodnar, G. Tang, R. B. Ness, G. Harger, and J. M. Roberts, "Periconceptional multivitamin use reduces the risk of preeclampsia," *American Journal of Epidemiology*, vol. 164, no. 5, pp. 470–477, 2006.

[53] I. Delbaere, H. Verstraelen, S. Goetgeluk, G. Martens, G. De Backer, and M. Temmerman, "Pregnancy outcome in primiparae of advanced maternal age," *European Journal of Obstetrics Gynecology & Reproductive Biology*, vol. 135, no. 1, pp. 41–46, 2007.

[54] S. C. Kalhan, "Protein metabolism in pregnancy," *American Journal of Clinical Nutrition*, vol. 71, no. 5, supplement, pp. 1249S–1255S, 2000.

[55] E. Sivan and G. Boden, "Free fatty acids, insulin resistance, and pregnancy," *Current Diabetes Reports*, vol. 3, no. 4, pp. 319–322, 2003.

[56] J. M. Potter and P. J. Nestel, "The hyperlipidemia of pregnancy in normal and complicated pregnancies," *American Journal of Obstetrics & Gynecology*, vol. 133, no. 2, pp. 165–170, 1979.

[57] J. Bonnar, L. Daly, and B. L. Sheppard, "Changes in the fibrinolytic system during pregnancy," *Seminars in Thrombosis and Hemostasis*, vol. 16, no. 3, pp. 221–229, 1990.

[58] D. M. Sherer and O. Abulafia, "Angiogenesis during implantation, and placental and early embryonic development," *Placenta*, vol. 22, no. 1, pp. 1–13, 2001.

[59] B. Sibai, G. Dekker, and M. Kupferminc, "Pre-eclampsia," *The Lancet*, vol. 365, no. 9461, pp. 785–799, 2005.

[60] J. M. Roberts and C. W. G. Redman, "Pre-eclampsia: more than pregnancy-induced hypertension," *The Lancet*, vol. 341, no. 8858, pp. 1447–1451, 1993.

[61] G. T. R. Manten, M. J. Sikkema, H. A. M. Voorbij, G. H. A. Visser, H. W. Bruinse, and A. Franx, "Risk factors for cardiovascular disease in women with a history of pregnancy complicated by preeclampsia or intrauterine growth restriction," *Hypertension in Pregnancy*, vol. 26, no. 1, pp. 39–50, 2007.

[62] V. A. Rodie, D. J. Freeman, N. Sattar, and I. A. Greer, "Pre-eclampsia and cardiovascular disease: metabolic syndrome of pregnancy?" *Atherosclerosis*, vol. 175, no. 2, pp. 189–202, 2004.

[63] J. Newstead, P. von Dadelszen, and L. A. Magee, "Preeclampsia and future cardiovascular risk," *Expert Review of Cardiovascular Therapy*, vol. 5, no. 2, pp. 283–294, 2007.

[64] R. J. Kaaja and I. A. Greer, "Manifestations of chronic disease during pregnancy," *Journal of the American Medical Association*, vol. 294, no. 21, pp. 2751–2757, 2005.

[65] I. A. Brosens, P. De Sutter, T. Hamerlynck, et al., "Endometriosis is associated with a decreased risk of pre-eclampsia," *Human Reproduction*, vol. 22, no. 6, pp. 1725–1729, 2007.

[66] F. Xue and K. B. Michels, "Intrauterine factors and risk of breast cancer: a systematic review and meta-analysis of current evidence," *Lancet Oncology*, vol. 8, no. 12, pp. 1088–1100, 2007.

[67] J. Vamecq and N. Latruffe, "Medical significance of peroxisome proliferator-activated receptors," *The Lancet*, vol. 354, no. 9173, pp. 141–148, 1999.

[68] J. M. Lehmann, L. B. Moore, T. A. Smith-Oliver, W. O. Wilkison, T. M. Willson, and S. A. Kliewer, "An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor γ (PPARγ)," *Journal of Biological Chemistry*, vol. 270, no. 22, pp. 12953–12956, 1995.

[69] H. Vosper, G. A. Khoudoli, T. L. Graham, and C. N. Palmer, "Peroxisome proliferator-activated receptor agonists, hyperlipidaemia, and atherosclerosis," *Pharmacology & Therapeutics*, vol. 95, no. 1, pp. 47–62, 2002.

[70] C. Chambrier, J.-P. Bastard, J. Rieusset, et al., "Eicosapentaenoic acid induces mRNA expression of peroxisome

proliferator-activated receptor γ," *Obesity Research*, vol. 10, no. 6, pp. 518–525, 2002.

[71] S. A. Kliewer, J. M. Lenhard, T. M. Willson, I. Patel, D. C. Morris, and J. M. Lehmann, "A prostaglandin J_2 metabolite binds peroxisome proliferator-activated receptor γ and promotes adipocyte differentiation," *Cell*, vol. 83, no. 5, pp. 813–819, 1995.

[72] G. Chinetti, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation," *Inflammation Research*, vol. 49, no. 10, pp. 497–505, 2000.

[73] L. Waite, E. C. Person, Y. Zhou, K.-H. Lim, T. S. Scanlan, and R. N. Taylor, "Placental peroxisome proliferator-activated receptor-γ is up-regulated by pregnancy serum," *Journal of Clinical Endocrinology & Metabolism*, vol. 85, no. 10, pp. 3808–3814, 2000.

[74] Q. Wang, H. Fujii, and G. T. Knipp, "Expression of PPAR and RXR isoforms in the developing rat and human term placentas," *Placenta*, vol. 23, no. 8-9, pp. 661–671, 2002.

[75] A. Tarrade, R. Lai Kuen, A. Malassiné, et al., "Characterization of human villous and extravillous trophoblasts isolated from first trimester placenta," *Laboratory Investigation*, vol. 81, no. 9, pp. 1199–1211, 2001.

[76] A. Yessoufou, A. Hichami, P. Besnard, K. Moutairou, and N. A. Khan, "Peroxisome proliferator-activated receptor α deficiency increases the risk of maternal abortion and neonatal mortality in murine pregnancy with or without diabetes mellitus: modulation of T cell differentiation," *Endocrinology*, vol. 147, no. 9, pp. 4410–4418, 2006.

[77] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[78] T. Fournier, L. Pavan, A. Tarrade, et al., "The role of PPAR-γ/RXR-α heterodimers in the regulation of human trophoblast invasion," *Annals of the New York Academy of Sciences*, vol. 973, pp. 26–30, 2002.

[79] Y.-J. Zhu, S. E. Crawford, V. Stellmach, et al., "Coactivator PRIP, the peroxisome proliferator-activated receptor-interacting protein, is a modulator of placental, cardiac, hepatic, and embryonic development," *Journal of Biological Chemistry*, vol. 278, no. 3, pp. 1986–1990, 2003.

[80] P. Antonson, G. U. Schuster, L. Wang, et al., "Inactivation of the nuclear receptor coactivator RAP250 in mice results in placental vascular dysfunction," *Molecular and Cellular Biology*, vol. 23, no. 4, pp. 1260–1268, 2003.

[81] V. Sapin, P. Dollé, C. Hindelang, P. Kastner, and P. Chambon, "Defects of the chorioallantoic placenta in mouse RXRα null fetuses," *Developmental Biology*, vol. 191, no. 1, pp. 29–41, 1997.

[82] N. Kubota, Y. Terauchi, H. Miki, et al., "PPARγ mediates high-fat diet-induced adipocyte hypertrophy and insulin resistance," *Molecular Cell*, vol. 4, no. 4, pp. 597–609, 1999.

[83] O. Wendling, P. Chambon, and M. Mark, "Retinoid X receptors are essential for early mouse development and placentogenesis," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 96, no. 2, pp. 547–551, 1999.

[84] L. L. H. Peeters, J.-L. Vigne, M. K. Tee, D. Zhao, L. Waite, and R. N. Taylor, "PPARγ represses VEGF expression in human endometrial cells: implications for uterine angiogenesis," *Angiogenesis*, vol. 8, no. 4, pp. 373–379, 2006.

[85] H. Bamba, S. Ota, A. Kato, C. Kawamoto, and K. Fujiwara, "Prostaglandins up-regulate vascular endothelial growth factor production through distinct pathways in differentiated U937 cells," *Biochemical and Biophysical Research Communications*, vol. 273, no. 2, pp. 485–491, 2000.

[86] V. Chintalgattu, G. S. Harris, S. M. Akula, and L. C. Katwa, "PPAR-γ agonists induce the expression of VEGF and its receptors in cultured cardiac myofibroblasts," *Cardiovascular Research*, vol. 74, no. 1, pp. 140–150, 2007.

[87] K. Yamakawa, M. Hosoi, H. Koyama, et al., "Peroxisome proliferator-activated receptor-γ agonists increase vascular endothelial growth factor expression in human vascular smooth muscle cells," *Biochemical and Biophysical Research Communications*, vol. 271, no. 3, pp. 571–574, 2000.

[88] T. Shalom-Barak, J. M. Nicholas, Y. Wang, et al., "Peroxisome proliferator-activated receptor γ controls *Muc1* transcription in trophoblasts," *Molecular and Cellular Biology*, vol. 24, no. 24, pp. 10661–10669, 2004.

[89] A. Tarrade, K. Schoonjans, J. Guibourdenche, et al., "PPARγ/RXRα heterodimers are involved in human CGβ synthesis and human trophoblast differentiation," *Endocrinology*, vol. 142, no. 10, pp. 4504–4514, 2001.

[90] W. T. Schaiff, I. Bildirici, M. Cheong, P. L. Chern, D. M. Nelson, and Y. Sadovsky, "Peroxisome proliferator-activated receptor-γ and retinoid X receptor signaling regulate fatty acid uptake by primary human placental Trophoblasts," *Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 7, pp. 4267–4275, 2005.

[91] M. Lappas, M. Permezel, and G. E. Rice, "Leptin and adiponectin stimulate the release of proinflammatory cytokines and prostaglandins from human placenta and maternal adipose tissue via nuclear factor-κB, peroxisomal proliferator-activated receptor-γ and extracellularly regulated kinase 1/2," *Endocrinology*, vol. 146, no. 8, pp. 3334–3342, 2005.

[92] K. Handschuh, J. Guibourdenche, M. Guesnon, I. Laurendeau, D. Evain-Brion, and T. Fournier, "Modulation of PAPP-A expression by PPARγ in human first trimester trophoblast," *Placenta*, vol. 27, supplement 1, pp. 127–134, 2006.

[93] R. L. Schild, W. T. Schaiff, M. G. Carlson, E. J. Cronbach, D. M. Nelson, and Y. Sadovsky, "The activity of PPARγ in primary human trophoblasts is enhanced by oxidized lipids," *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 3, pp. 1105–1110, 2002.

[94] R. Asami-Miyagishi, S. Iseki, M. Usui, K. Uchida, H. Kubo, and I. Morita, "Expression and function of PPARγ in rat placental development," *Biochemical and Biophysical Research Communications*, vol. 315, no. 2, pp. 497–501, 2004.

[95] E. Capobianco, A. Jawerbaum, M. C. Romanini, et al., "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 and peroxisome proliferator-activated receptor γ (PPARγ) levels in term placental tissues from control and diabetic rats: modulatory effects of a PPARγ agonist on nitridergic and lipid placental metabolism," *Reproduction, Fertility and Development*, vol. 17, no. 4, pp. 423–433, 2005.

[96] M. Lappas, M. Permezel, H. M. Georgiou, and G. E. Rice, "Regulation of proinflammatory cytokines in human gestational tissues by peroxisome proliferator-activated receptor-γ: effect of 15-deoxy-$\Delta^{12,14}$-PGJ_2 and troglitazone," *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 10, pp. 4667–4672, 2002.

[97] K. W. Marvin, R. L. Eykholt, J. A. Keelan, T. A. Sato, and M. D. Mitchell, "The 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 receptor,

peroxisome proliferator activated receptor-γ (PPARγ) is expressed in human gestational tissues and is functionally active in JEG3 choriocarcinoma cells," *Placenta*, vol. 21, no. 4, pp. 436–440, 2000.

[98] L. R. Dunn-Albanese, W. E. Ackerman IV, Y. Xie, J. D. Iams, and D. A. Kniss, "Reciprocal expression of peroxisome proliferator-activated receptor-γ and cyclooxygenase-2 in human term parturition," *American Journal of Obstetrics & Gynecology*, vol. 190, no. 3, pp. 809–816, 2004.

[99] E. B. E. Berry, R. Eykholt, R. J. A. Helliwell, R. S. Gilmour, M. D. Mitchell, and K. W. Marvin, "Peroxisome proliferator-activated receptor isoform expression changes in human gestational tissues with labor at term," *Molecular Pharmacology*, vol. 64, no. 6, pp. 1586–1590, 2003.

[100] B. M. Forman, P. Tontonoz, J. Chen, R. P. Brun, B. M. Spiegelman, and R. M. Evans, "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 is a ligand for the adipocyte determination factor PPARγ," *Cell*, vol. 83, no. 5, pp. 803–812, 1995.

[101] R. L. Schild, C. M. Sonnenberg-Hirche, W. T. Schaiff, I. Bildirici, D. M. Nelson, and Y. Sadovsky, "The kinase p38 regulates peroxisome proliferator activated receptor- in human trophoblasts," *Placenta*, vol. 27, no. 2-3, pp. 191–199, 2006.

[102] S. S.-T. Lee and F. J. Gonzalez, "Targeted disruption of the peroxisome proliferator-activated receptor α gene, PPARα," *Annals of the New York Academy of Sciences*, vol. 804, no. 1, pp. 524–529, 1996.

[103] G. B. Dealtry, M. K. O'Farrell, and N. Fernandez, "The Th2 cytokine environment of the placenta," *International Archives of Allergy and Immunology*, vol. 123, no. 2, pp. 107–119, 2000.

[104] J. C. Huang, W. S. Wun, J. S. Goldsby, I. C. Wun, D. Noorhasan, and K. K. Wu, "Stimulation of embryo hatching and implantation by prostacyclin and peroxisome proliferator-activated receptor δ activation: implication in IVF," *Human Reproduction*, vol. 22, no. 3, pp. 807–814, 2007.

[105] H. Lim and S. K. Dey, "PPARδ functions as a prostacyclin receptor in blastocyst implantation," *Trends in Endocrinology & Metabolism*, vol. 11, no. 4, pp. 137–142, 2000.

[106] M. Lappas, M. Permezel, H. M. Georgiou, and G. E. Rice, "Nuclear factor κ B regulation of proinflammatory cytokines in human gestational tissues in vitro," *Biology of Reproduction*, vol. 67, no. 2, pp. 668–673, 2002.

[107] S. A. Törnblom, F. A. Patel, B. Byström, et al., "15-Hydroxyprostaglandin dehydrogenase and cyclooxygenase 2 messenger ribonucleic acid expression and immunohistochemical localization in human cervical tissue during term and preterm labor," *Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 6, pp. 2909–2915, 2004.

[108] M. Lappas, M. Permezel, and G. E. Rice, "15-deoxy-$\Delta^{12,14}$-rostaglandin J_2 and troglitazone regulation of the release of phospholipid metabolites, inflammatory cytokines and proteases from guman gestational tissues," *Placenta*, vol. 27, no. 11-12, pp. 1060–1072, 2006.

[109] E. T. McKinney, R. Shouri, R. S. Hunt, R. A. Ahokas, and B. M. Sibai, "Plasma, urinary, and salivary 8-epi-prostaglandin $F_{2\alpha}$ levels in normotensive and preeclamptic pregnancies," *American Journal of Obstetrics & Gynecology*, vol. 183, no. 4, pp. 874–877, 2000.

[110] D. B. Klinkner, H. J. Lim, E. Y. Strawn Jr., K. T. Oldham, and T. L. Sander, "An in vivo murine model of rosiglitazone use in pregnancy," *Fertility and Sterility*, vol. 86, no. 4, supplement 1, pp. 1074–1079, 2006.

[111] L. Waite, R. E. Louie, and R. N. Taylor, "Circulating activators of peroxisome proliferator-activated receptors are reduced in preeclamptic pregnancy," *Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 2, pp. 620–626, 2005.

[112] E. C. Tok, D. Ertunc, O. Bilgin, E. M. Erdal, M. Kaplanoglu, and S. Dilek, "PPAR-γ2 Pro12Ala polymorphism is associated with weight gain in women with gestational diabetes mellitus," *European Journal of Obstetrics Gynecology & Reproductive Biology*, vol. 129, no. 1, pp. 25–30, 2006.

[113] A. Jawerbaum, E. Capobianco, C. Pustovrh, et al., "Influence of peroxisome proliferator-activated receptor γ activation by its endogenous ligand 15-deoxy$\Delta^{12,14}$ prostaglandin J_2 on nitric oxide production in term placental tissues from diabetic women," *Molecular Human Reproduction*, vol. 10, no. 9, pp. 671–676, 2004.

[114] L. Capparuccia, D. Marzioni, A. Giordano, et al., "PPARγ expression in normal human placenta, hydatidiform mole and choriocarcinoma," *Molecular Human Reproduction*, vol. 8, no. 6, pp. 574–579, 2002.

[115] V. A. Rodie, A. Young, F. Jordan, N. Sattar, I. A. Greer, and D. J. Freeman, "Human placental peroxisome proliferator-activated receptor δ and γ expression in healthy pregnancy and in preeclampsia and intrauterine growth restriction," *Journal of the Society for Gynecologic Investigation*, vol. 12, no. 5, pp. 320–329, 2005.

[116] A. Meirhaeghe, C. A. G. Boreham, L. J. Murray, et al., "A possible role for the *PPARG* Pro12Ala polymorphism in preterm birth," *Diabetes*, vol. 56, no. 2, pp. 494–498, 2007.

[117] J. R. Higgins and M. de Swiet, "Blood-pressure measurement and classification in pregnancy," *The Lancet*, vol. 357, no. 9250, pp. 131–135, 2001.

[118] A. P. Mackay, C. J. Berg, and H. K. Atrash, "Pregnancy-related mortality from preeclampsia and eclampsia," *Obstetrics & Gynecology*, vol. 97, no. 4, pp. 533–538, 2001.

[119] R. N. Taylor, C. J. M. de Groot, Y. K. Cho, and K.-H. Lim, "Circulating factors as markers and mediators of endothelial cell dysfunction in preeclampsia," *Seminars in Reproductive Endocrinology*, vol. 16, no. 1, pp. 17–31, 1998.

[120] Y. Hamai, T. Fujii, T. Yamashita, et al., "Evidence for an elevation in serum interleukin-2 and tumor necrosis factor-α levels before the clinical manifestations of preeclampsia," *American Journal of Reproductive Immunology*, vol. 38, no. 2, pp. 89–93, 1997.

[121] K.-A. Wathén, E. Tuutti, U.-H. Stenman, et al., "Maternal serum-soluble vascular endothelial growth factor receptor-1 in early pregnancy ending in preeclampsia or intrauterine growth retardation," *Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 1, pp. 180–184, 2006.

[122] S.-M. Yie, L.-H. Li, Y.-M. Li, and C. Librach, "HLA-G protein concentrations in maternal serum and placental tissue are decreased in preeclampsia," *American Journal of Obstetrics & Gynecology*, vol. 191, no. 2, pp. 525–529, 2004.

[123] R. Thadhani, W. P. Mutter, M. Wolf, et al., "First trimester placental growth factor and soluble FMS-like tyrosine kinase 1 and risk for preeclampsia," *Journal of Clinical Endocrinology & Metabolism*, vol. 89, no. 2, pp. 770–775, 2004.

[124] D. V. Coonrod, D. E. Hickok, K. Zu, T. R. Easterling, and J. R. Daling, "Risk factors for preeclampsia in twin pregnancies: a population-based cohort study," *Obstetrics & Gynecology*, vol. 85, no. 5, part 1, pp. 645–650, 1995.

[125] M. J. McMahon, C. V. Ananth, and R. M. Liston, "Gestational diabetes mellitus: risk factors, obstetric complications and infant outcomes," *Journal of Reproductive Medicine for the Obstetrician and Gynecologist*, vol. 43, no. 4, pp. 372–378, 1998.

[126] M. Tanaka, G. Jaamaa, M. Kaiser, et al., "Racial disparity in hypertensive disorders of pregnancy in New York state: a 10-year longitudinal population-based study," *American Journal of Public Health*, vol. 97, no. 1, pp. 163–170, 2007.

[127] B. A. Beamer, C.-J. Yen, R. E. Andersen, et al., "Association of the Pro12Ala variant in the peroxisome proliferator- activated receptor-γ2 gene with obesity in two Caucasian populations," *Diabetes*, vol. 47, no. 11, pp. 1806–1808, 1998.

[128] S. J. Hasstedt, Q.-F. Ren, K. Teng, and S. C. Elbein, "Effect of the peroxisome proliferator-activated receptor-γ2 Pro12Ala variant on obesity, glucose homeostasis, and blood pressure in members of familial type 2 diabetic kindreds," *Journal of Clinical Endocrinology & Metabolism*, vol. 86, no. 2, pp. 536–541, 2001.

[129] W.-D. Li, J. H. Lee, and R. A. Price, "The peroxisome proliferator-activated receptor γ2 Pro12Ala mutation is associated with early onset extreme obesity and reduced fasting glucose," *Molecular Genetics and Metabolism*, vol. 70, no. 2, pp. 159–161, 2000.

[130] S. S. Deeb, L. Fajas, M. Nemoto, et al., "A Pro12Ala substitution in PPARγ2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity," *Nature Genetics*, vol. 20, no. 3, pp. 284–287, 1998.

[131] J. Laasanen, S. Heinonen, M. Hiltunen, A. Mannermaa, and M. Laakso, "Polymorphism in the peroxisome proliferator-activated receptor-γ gene in women with preeclampsia," *Early Human Development*, vol. 69, no. 1-2, pp. 77–82, 2002.

[132] C. A. Hubel, "Oxidative stress in the pathogenesis of preeclampsia," *Proceedings of the Society for Experimental Biology and Medicine*, vol. 222, no. 3, pp. 222–235, 1999.

[133] B. W. Arbogast, S. C. Leeper, R. D. Merrick, K. E. Olive, and R. N. Taylor, "Which plasma factors bring about disturbance of endothelial function in pre-eclampsia?" *The Lancet*, vol. 343, no. 8893, pp. 340–341, 1994.

[134] C. W. G. Redman and I. L. Sargent, "Pre-eclampsia, the placenta and the maternal systemic inflammatory response—a review," *Placenta*, vol. 24, supplement 1, pp. S21–S27, 2003.

[135] M. J. Reginato, S. L. Krakow, S. T. Bailey, and M. A. Lazar, "Prostaglandins promote and block adipogenesis through opposing effects on peroxisome proliferator-activated receptor γ," *Journal of Biological Chemistry*, vol. 273, no. 4, pp. 1855–1858, 1998.

[136] P. L. Ogburn Jr., S. B. Johnson, P. P. Williams, and R. T. Holman, "Levels of free fatty acids and arachidonic acid in pregnancy and labor," *Journal of Laboratory and Clinical Medicine*, vol. 95, no. 6, pp. 943–949, 1980.

[137] P. L. Ogburn Jr., P. P. Williams, S. B. Johnson, and R. T. Holman, "Serum arachidonic acid levels in normal and preeclamptic pregnancies," *American Journal of Obstetrics & Gynecology*, vol. 148, no. 1, pp. 5–9, 1984.

[138] C. J. M. de Groot, J. T. Murai, J.-L. Vigne, and R. N. Taylor, "Eicosanoid secretion by human endothelial cells exposed to normal pregnancy and preeclampsia plasma in vitro," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 58, no. 2, pp. 91–97, 1998.

[139] D. Hornung, I. P. Ryan, V. A. Chao, J.-L. Vigne, E. D. Schriock, and R. N. Taylor, "Immunolocalization and regulation of the chemokine RANTES in human endometrial and endometriosis tissues and cells," *Journal of Clinical Endocrinology & Metabolism*, vol. 82, no. 5, pp. 1621–1628, 1997.

[140] S. Blackburn and D. Loper, *Maternal Fetal and Neonatal Physiology: A Clinical Perspective*, Harcourt Brace Jovanovic, Philadelphia, Pa, USA, 1992.

[141] W. W. Hay Jr., "Placental transport of nutrients to the fetus," *Hormone Research*, vol. 42, no. 4-5, pp. 215–222, 1994.

[142] L. Jovanovic and D. J. Pettitt, "Gestational diabetes mellitus," *Journal of the American Medical Association*, vol. 286, no. 20, pp. 2516–2518, 2001.

[143] C. B. Rudra, T. K. Sorensen, W. M. Leisenring, E. Dashow, and M. A. Williams, "Weight characteristics and height in relation to risk of gestational diabetes mellitus," *American Journal of Epidemiology*, vol. 165, no. 3, pp. 302–308, 2007.

[144] F. Galtier-Dereure, C. Boegner, and J. Bringer, "Obesity and pregnancy: complications and cost," *American Journal of Clinical Nutrition*, vol. 71, no. 5, supplement, pp. 1242S–1248S, 2000.

[145] P. M. Catalano, "Increasing maternal obesity and weight gain during pregnancy: the obstetric problems of plentitude," *Obstetrics & Gynecology*, vol. 110, no. 4, pp. 743–744, 2007.

[146] C. J. P. Jones and H. Fox, "Placental changes in gestational diabetes. An ultrastructural study," *Obstetrics & Gynecology*, vol. 48, no. 3, pp. 274–280, 1976.

[147] A. Åberg, H. Rydhstroem, and A. Frid, "Impaired glucose tolerance associated with adverse pregnancy outcome: a population-based study in southern Sweden," *American Journal of Obstetrics & Gynecology*, vol. 184, no. 2, pp. 77–83, 2001.

[148] D. B. Carr, K. M. Utzschneider, R. L. Hull, et al., "Gestational diabetes mellitus increases the risk of cardiovascular disease in women with a family history of type 2 diabetes," *Diabetes Care*, vol. 29, no. 9, pp. 2078–2083, 2006.

[149] H. Lee, H. C. Jang, H. K. Park, and N. H. Cho, "Early manifestation of cardiovascular disease risk factors in offspring of mothers with previous history of gestational diabetes mellitus," *Diabetes Research and Clinical Practice*, vol. 78, no. 2, pp. 238–245, 2007.

[150] M. Morifuji, C. Sanbongi, and K. Sugiura, "Dietary soya protein intake and exercise training have an additive effect on skeletal muscle fatty acid oxidation enzyme activities and mRNA levels in rats," *British Journal of Nutrition*, vol. 96, no. 3, pp. 469–475, 2006.

[151] C. Zhang, S. Liu, C. G. Solomon, and F. B. Hu, "Dietary fiber intake, dietary glycemic load, and the risk for gestational diabetes mellitus," *Diabetes Care*, vol. 29, no. 10, pp. 2223–2230, 2006.

[152] E. Oken, Y. Ning, S. L. Rifas-Shiman, J. S. Radesky, J. W. Rich-Edwards, and M. W. Gillman, "Associations of physical activity and inactivity before and during pregnancy with glucose tolerance," *Obstetrics & Gynecology*, vol. 108, no. 5, pp. 1200–1207, 2006.

[153] T. L. Weissgerber, L. A. Wolfe, G. A. L. Davies, and M. F. Mottola, "Exercise in the prevention and treatment of maternal-fetal disease: a review of the literature," *Applied Physiology, Nutrition and Metabolism*, vol. 31, no. 6, pp. 661–674, 2006.

[154] H. Leipold, M. Knoefler, C. Gruber, A. Huber, P. Haslinger, and C. Worda, "Peroxisome proliferator-activated receptor γ coactivator-1α gene variations are not associated with gestational diabetes mellitus," *Journal of the Society for Gynecologic Investigation*, vol. 13, no. 2, pp. 104–107, 2006.

[155] S. Fukuen, M. Iwaki, A. Yasui, M. Makishima, M. Matsuda, and I. Shimomura, "Sulfonylurea agents exhibit peroxisome proliferator-activated receptor γ agonistic activity," *Journal of Biological Chemistry*, vol. 280, no. 25, pp. 23653–23659, 2005.

[156] O. Langer, D. L. Conway, M. D. Berkus, E. M.-J. Xenakis, and O. Gonzales, "A comparison of glyburide and insulin in women with gestational diabetes mellitus," *New England Journal of Medicine*, vol. 343, no. 16, pp. 1134–1138, 2000.

[157] C. Ferretti, L. Bruni, V. Dangles-Marie, A. P. Pecking, and D. Bellet, "Molecular circuits shared by placental and cancer cells, and their implications in the proliferative, invasive and migratory capacities of trophoblasts," *Human Reproduction Update*, vol. 13, no. 2, pp. 121–141, 2007.

[158] D. P. Hewitt, P. J. Mark, and B. J. Waddell, "Placental expression of peroxisome proliferator-activated receptors in rat pregnancy and the effect of increased glucocorticoid exposure," *Biology of Reproduction*, vol. 74, no. 1, pp. 23–28, 2006.

[159] W. T. Schaiff, F. F. Knapp Jr., Y. Barak, T. Biron-Shental, D. M. Nelson, and Y. Sadovsky, "Ligand-activated peroxisome proliferator activated receptor γ alters placental morphology and placental fatty acid uptake in mice," *Endocrinology*, vol. 148, no. 8, pp. 3625–3634, 2007.

[160] J. A. Keelan, R. J. A. Helliwell, B. E. Nijmeijer, et al., "15-deoxy-$\Delta^{12,14}$-prostaglandin J_2-induced apoptosis in amnion-like WISH cells," *Prostaglandins & Other Lipid Mediators*, vol. 66, no. 4, pp. 265–282, 2001.

[161] H. Hasegawa, H. Takano, Y. Zou, et al., "Pioglitazone, a peroxisome proliferator-activated receptor γ activator, ameliorates experimental autoimmune myocarditis by modulating Th1/Th2 balance," *Journal of Molecular and Cellular Cardiology*, vol. 38, no. 2, pp. 257–265, 2005.

[162] S. Dasgupta, A. Roy, M. Jana, D. M. Hartley, and K. Pahan, "Gemfibrozil ameliorates relapsing-remitting experimental autoimmune encephalomyelitis independent of peroxisome proliferator-activated receptor-α," *Molecular Pharmacology*, vol. 72, no. 4, pp. 934–946, 2007.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 758562, 9 pages
doi:10.1155/2008/758562

Review Article

Placental Implications of Peroxisome Proliferator-Activated Receptors in Gestation and Parturition

Valerie Borel,[1,2] Denis Gallot,[1,3] Geoffroy Marceau,[1,2] Vincent Sapin,[1,2] and Loïc Blanchon[1,2]

[1] *Université d'Auvergne, JE 2447, ARDEMO, 63000 Clermont-Ferrand, France*
[2] *INSERM, U.384, Laboratoire de Biochimie, Faculté de Médecine, 63000 Clermont-Ferrand, France*
[3] *CHU Clermont-Ferrand, Maternité, Hôtel-Dieu, 63000 Clermont-Ferrand, France*

Correspondence should be addressed to Vincent Sapin, vincent.sapin@u-clermont1.fr

Received 4 June 2007; Revised 13 August 2007; Accepted 26 October 2007

Recommended by Pascal Froment

The placenta is a transitory structure indispensable for the proper development of the embryo and fetus during mammalian gestation. Like other members of the nuclear receptor family, the peroxisome proliferator-activated receptors (PPARs) are known to be involved in the physiological and pathological events occurring during the placentation. This placental involvement has been recently reviewed focusing on the early stages of placental development (implantation and invasion, etc.), mouse PPARs knockout phenotypes, and cytotrophoblast physiology. In this review, we describe the placental involvement of PPARs (e.g., fat transport and metabolism, etc.) during the late stages of gestation and in the amniotic membranes, highlighting their roles in the inflammation process (e.g., chorioamnionitis), metabolic disorders (e.g., diabetes), and parturition.

1. THE PEROXISOME PROLIFERATOR-ACTIVATED RECEPTORS (PPARs)

1.1. Nomenclature and structure

Discovered in 1990, PPARs are known for their biological role in inducing the proliferation of peroxisomes in rodents [1]. They are transcription factors belonging to the ligand-activated nuclear hormone receptor superfamily [2] and have been identified in different species such as the xenopus, mouse, rat, and humans. In all these species, PPARs present three isotypes encoded by distinct single-copy genes: PPARα (NR1C1), PPARβ/δ (also called NUC1 or NR1C2), and PPARγ (NR1C3), located on chromosomes 15, 17, 6 in the mouse and chromosomes 22, 6, 3 in humans, respectively. The PPARγ gene alternative promoters give rise to three different isoforms named γ1, γ2, and γ3 which differ at their 5′ends (see Figure 1(a)) [3]. PPAR α, β, γ1/γ3, γ2 translation produces proteins of 468, 441, 475, and 505 amino acids, respectively, with a molecular weight of 49 to 56 kDa [4]. By performing multiple PPAR nucleotide/protein alignments of PPARs in different species, a strong interspecies identity (human, mouse, rat, bovine, ≈90%) has been established, illustrating a strong evolutionary conservation among species by derivation from a common ancestor (Table 1). PPARγ shows the highest conservation in terms of cDNA and proteins.

Like several other members of the nuclear receptor superfamily, PPARs possess the typical structure organised in six domains named A to F (see Figure 1(b)) [5]. Domain C (DBD: DNA binding domain) contains two zinc fingers and allows promoter target gene interaction and dimerization with its preferential nuclear receptor: retinoid X receptor (RXR). The PPAR/RXR heterodimer binds to the target gene promoter response element named peroxisome proliferator response element (PPRE) which is made up of two half site AGGTCA separated by one or two nucleotides (also called DR1 or DR2 for direct repeat 1 or 2) and a 5′ extension A (A/T) CT. Domain E/F allows ligand binding and contains a ligand-dependent transactivation function called AF2 (activating function 2). It is involved in dimerization and interaction with cofactors.

1.2. PPAR ligands

As with the other nuclear receptors, the binding of the ligand is a key step in the control of PPAR transcriptional activity. In

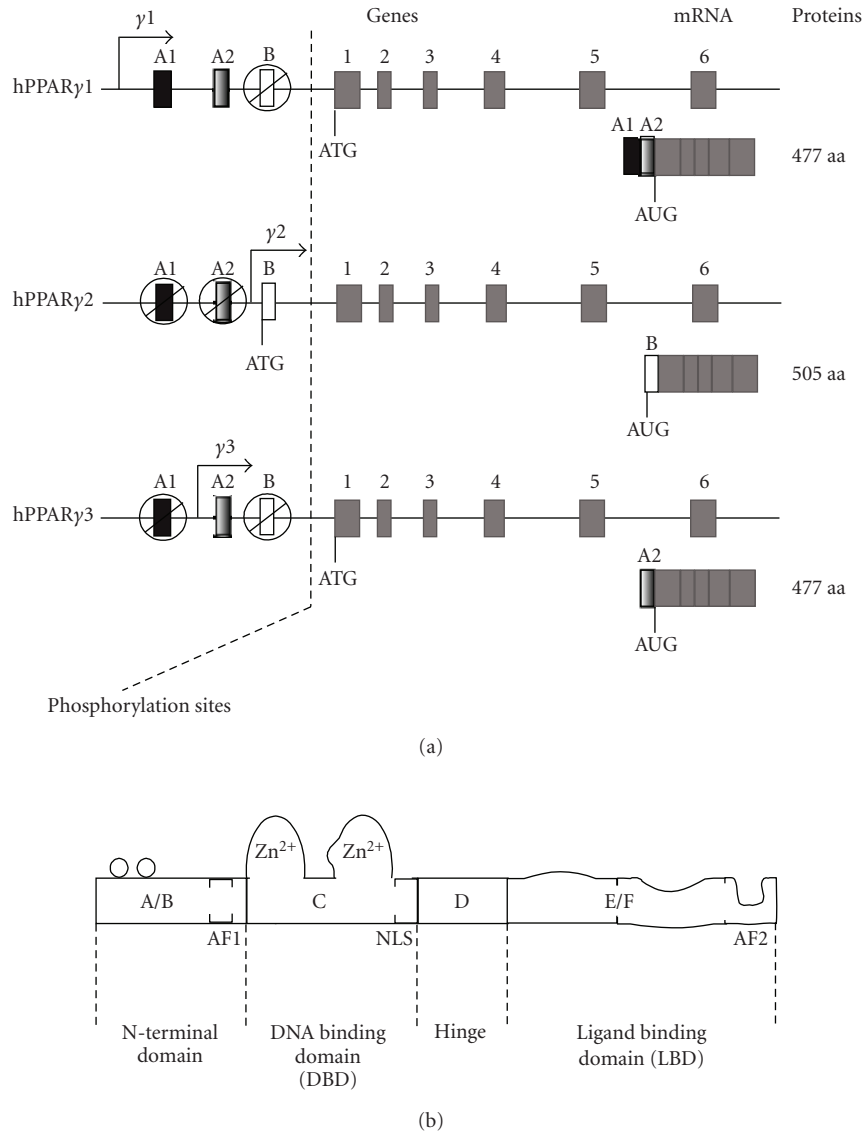


Figure 1: (a) Schematic representation of PPARγ genes, mRNA, and proteins. The 5′exons A1, A2, B can be alternatively spliced to give rise to the different PPARγ isoforms. The boxes 1 to 6 correspond to exons which are common to PPARγ1, γ2, γ3 genes. ATG is the initiation transcription site. The molecular weight of these isoforms ranges from 49 to 56 kDa. (b) Schematic representation of typical nuclear receptor structure. AF1: activating function 1 (ligand-independent function), AF2: activating function 2 (ligand-dependent function), NLS: nuclear signal localization.

the absence of a ligand, corepressors and histone deacetylases (HDAC) bind to PPARs and inhibit the transcription activation of target genes. PPAR ligands have the ability to dissociate the corepressor complexes from the PPAR/RXR heterodimer, allowing the binding of the coactivators in order to initiate and activate transcription.

There are two kinds of ligands for the PPARs: natural and synthetic. Among the natural ligands the monounsaturated fatty acids (FA) (e.g., oleic acid) and the polyunsaturated fatty acids (PUFA) (e.g., linoleic acid, linolenic acid, and arachidonic acid) are described as ligands for PPARα, PPARβ, and PPARγ. They act with concentrations consistent with those found in human serum [6]. The different PUFA metabolites: 8(S)- and 15-hydroxyeicosatetraenoic acid (8(S)- and 15-HETE), leukotriene B4 (LTB4), 9- and 13-hydroxyoctadedienoic acid (9-HODE and 13-HODE) and 15-deoxy-$\Delta^{12,14}$-prostaglandin J2 (PGJ2) are potent selective activators of PPARα and PPARγ. Some oxidized low-density lipoproteins (LDLs), oxidized alkyphospholipids, nitrolinolenic acid, and prostaglandin metabolites can also activate PPARγ [7]. Recently, it has been demonstrated that P450 eicosanoids are potent PPARα and PPARγ ligands [8]. Indeed, Ng et al. [8] have shown that P450 catalysed arachidonic acid metabolites like 20-hydroxyeicosatetraenoic acid (20-HETE) or 11, 12-epoxyeicosatrienoic acid (11, 12-EET) can activate PPARα and PPARγ. These ligands induce PPAR binding to PPRE and can modify the expression of PPARα responsive genes like apoA-I or apoA-II

in the same way than synthetic ligands. Thus the finely regulated conversion of PUFAs to eicosanoids through either the lipoxygenase, cyclooxygenase, or cytochrome P450 monooxygenase pathways may provide a mechanism for the differential regulation of PPARα and PPARγ and their respective target genes. PPARβ can be activated by different types of eicosanoids including prostaglandinA1 (PGA1) and prostaglandin D2 (PGD2). Many synthetic ligands exist and have been used in PPAR work. These ligands include prostaglandin 12 analogs, pirinixic acid (Wy-14643) for PPARα, hypolipidemic and hypoglycemic agents (nonthiazolidinedione) for PPARβ, and thiazolidinediones (e.g., rosiglitasone, troglitazone) for PPARγ [2].

2. PPAR EXPRESSION PATTERNS

The adult PPAR expression patterns have been extensively established at the mRNA and protein levels in several species (Table 2) [8, 9]. Several studies conducted during mammalian gestation have established the placenta as an important expression site of the different PPARs isoforms. Our review will focus only on term placental expression and on the amniotic/fetal membranes. The placental dynamic expression of the 3 PPARs during early and midgestation (of mouse, rat, and human) is well described in Fournier et al., 2007 [4]. In rat placenta, all three PPAR isoforms are ubiquitously expressed from 11 days postcoitum (dpc) [10]. Both PPARβ/δ and PPARγ are expressed after 8.5 dpc in mouse placenta. By immunohistochemistry and RT-PCR, the three PPAR isoforms are been shown to be expressed in the villous trophoblastic cells and syncytiotrophoblasts of the human term placenta [4]. To extend the previously published results [11] and to assess the potential importance of PPAR proteins in fetal membranes, RT-PCR and immunohistochemistry experiments were performed on human term placental samples. The three PPARs are present in total placenta, amnion, chorion, and in amnion-derived WISH epithelial cell line at the mRNA (see Figure 2(a)) and protein levels (see Figure 2(b)). The expression of PPARα and PPARγ seems to be weaker than that observed for PPARβ/δ. In addition, a greater amplification of the PPARγ cDNA is obtained in chorion than in amnion, where PPARγ is almost undetectable.

3. IMPLICATIONS OF PPARs IN PLACENTA AND FETAL MEMBRANES

3.1. Placental and amniotic presence of PPARs ligands

The lipids of human amnion and chorion are enriched in the essential fatty acid arachidonic acid, which is the precursor of all the prostaglandins of the 2 series [13]. Sixty-six percent of the arachidonic acid of the human fetal membranes are available in the glycerophospholipids of these tissues and can easily be converted into PGD_2 [14]. The placenta produces considerable amounts of PGD2 [15]. The enzymes necessary to convert PGD_2 into prostaglandin J2 (PGJ2) are present and coexpressed with PPARγ in placenta. 15-Deoxy-$\Delta^{12,14}$-PGJ2 (15dPGJ2) and its precursor PGD2 are present in amniotic fluid at concentrations that do not exceed 3 nM [16]. However, this amniotic fluid concentration cannot be an exact representation of the physiological placental reality for PPARs ligands because the nuclear concentration is not measured. The maternal blood may also be a source of PPAR ligands for the human placenta and the fetal membranes. It has been established that a heat-stable compound (not a protein, but rather a prostanoid or a fatty acid) is detected in maternal blood serum and is able to activate the PPARγ [17]. The presence of classical and new PPARs ligands (e.g., P450 eicosanoids, PUFA metabolites) in placenta and fetal membranes suggests that they could activate PPAR, induce PPAR binding to PPRE, and modify the expression of PPAR target genes; but this hypothesis has to be confirmed by further analysis, based on PPARs activation in other organs. For example, PUFAs, such as and eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), increased PPARγ mRNA expression and binding to PPRE in renal tubular epithelial cell line (HK-2). Furthermore, they downregulate LPS-induced activation of NF-κB via a PPARγ-dependent pathway in HK-2 cells [18]. Another example showed that PGD2 is among the most abundantly produced prostaglandins in synovial fluid by synovial fibroblasts [19]. It can be converted into PGJ2. It has been demonstrated that PPARγ ligands (15dPGJ2) inhibit IL-1β–induced production of nitric oxide (NO) and matrix metalloproteinase-13 (MMP-13) in chondrocytes. This inhibition was PPARγ-dependent and occurred at the transcriptional level, through repression of NF-κB signalling [20]. These two examples support a role of PPAR ligands in fetal membranes.

3.2. Fundamental implications of PPARs during early placentation

As a determining result, the knockout of the PPARγ in mice [21] yielded the first findings indicating the importance of this factor in early embryonic and perinatal development. These results are concomitant with those obtained by the generation of RXRα or β null mice (PPARγ partner in the functional heterodimer), also showing an embryonic lethality explained by the lack of generation of a functional labyrinthine zone [22]. Furthermore, complementary studies conducted by the inactivation of PPARγ coactivators or coregulators, such as peroxisome proliferators activator receptor-binding protein (PBP) and peroxisome proliferator-activated receptor-interacting protein (PRIP), also lead to severe placental dysfunction, such as inadequate vascularisation of the structure [23–25]. Recently, Barak et al. also demonstrated that the inactivation of PPARβ/δ led to the formation of abnormal gaps and a thinner but fully differentiated vascular structure in the placentodecidual interface [26]. These results establish the nonredundant roles of PPARγ and PPARβ/δ in early mouse placental development. By contrast, the inactivation of PPARα has no effect on placental formation or on the developing foetus and by the way theirs possible roles during pregnancy had to be clarified [2]. In humans, the studies are almost exclusively

Table 1: Percentage of nucleotide and amino acid identity between the human, mouse, rat, and bovine PPAR sequences. No PPARγ3 alignment was carried out owing to lack of data on different species. The different sequences came from Ensembl and were aligned with Genomatix software.

		cDNA homology (%)			Protein homology (%)		
		Mouse	Rat	Bovine	Mouse	Rat	Bovine
Human relative identity percent	PPARα	44	64	72	92	92	94
	PPARβ	60	69	75	92	91	95
	PPARγ1	79	84	78	98	97	97
	PPARγ2	86	86	88	96	95	95

Table 2: Summary of PPAR expression patterns.

(a) Global expression pattern			
Gene	Species	Expression localization	References
PPARα	Rodents	Cardiomyocytes	[6, 8]
		Hepatocytes	
	Human	Heart	[6, 8]
		Kidney	
		Large intestine	
		Leydig and seminiferous tubule cells	[12]
		Liver	[6, 8]
		Skeletal muscle	
		Uterus	[12]
		Ovary (Theca and stroma cells)	
PPARβ	Rodents	Ubiquitous	[6, 8]
	Human	Ubiquitous	[6, 8]
PPARγ	Rodents	Brown and white adipose tissue	[6, 8]
		Lymphoid organs	
		Retina	
		Skeletal muscle	
		Uterus	[12]
		Granulosa cells, corpus luteum	
	Human	Colon	[6, 8]
		Kidney	
		Liver	
		Skeltal muscle	
		Vascular endothelium	
		White adipose tissue	
		Sertoli cells	[12]
		Uterus	
		Granulosa cells	
(B) Placental expression pattern			
PPARα, β, γ	Rodents	Term placenta	[4, 9]
	Human	Villous trophoblastic cells and syncytiotrophoblasts	[4, 9, 10]
		Amnion, chorion, and amnion derived-WISH cell line	

focused on the PPARγ roles during early placentation. It has been clearly established that all three PPARs can stimulate or inhibit the differentiation and/or proliferation of the villous cytotrophoblasts into syncytiotrophoblasts and the synthesis of chorionic gonadotrophic hormone, and may hamper extravillous trophoblastic cell invasion (for more details, see Fournier et al., 2007 [4]).

3.3. Roles of PPARs in the uptake and transport of trophoblastic lipids

As one of the first functions described for PPARγ in other tissues, trophoblastic lipid uptake and accumulation are also regulated in part by this factor [27]. The PPARγ ligands seem to increase the uptake and accumulation of the fatty acids in

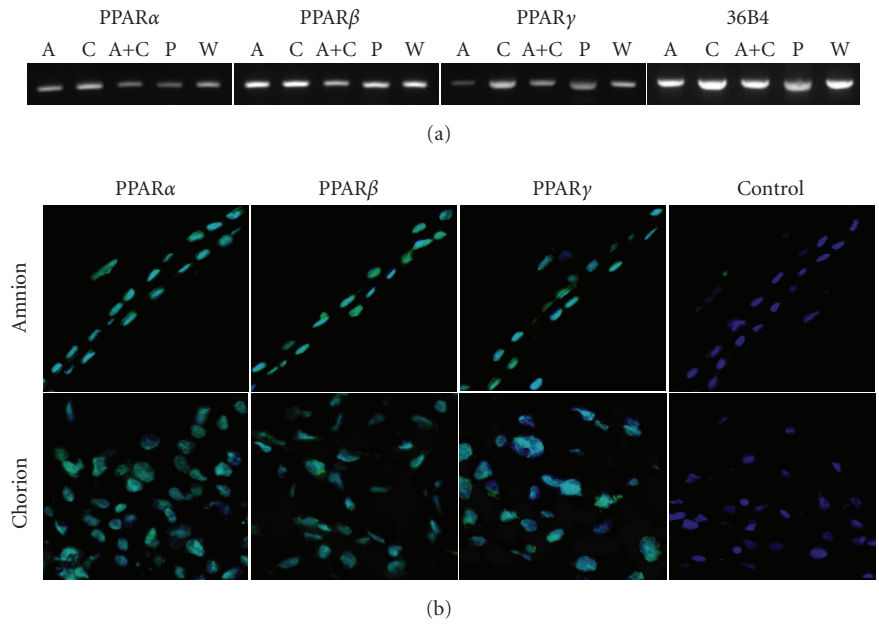


Figure 2: PPARs expression in term placenta and amniotic membrances. (a) RT-PCR assays of PPARα, PPARβ, and PPARγ mRNA in amnion, chorion, placenta, and WISH cells. PCR products were analyzed on 1.8% agarose gel and stained with ethidium bromide. 36B4 corresponds to the housekeeping gene. A: Amnion, C: Chorion, A+C: Amnino+Chorion, P: Total placenta, W: WISH cells. (b) PPARs immunostaining of amnion and chorion. Note that all PPARs are expressed in nucleus. Magnification: x200.

human placenta [28]. This regulation is associated with an enhanced expression of adipophilin (fat droplet-associated protein) and fatty acid transport proteins (1 and 4) in human trophoblasts [28–30]. These results were confirmed recently by the in vivo activation of PPARγ by its agonist rosiglitazone in mice, which also leads to the enhancement of the previous described genes plus two new ones involved in the lipid transport: S3-12 (plasma associated protein) and myocardial lipid droplet protein/MLDP [27]. Taken together, these results confirm the results obtained on PPARγ-null mutants: the absence of the lipid droplets normally present around the fetal vessels in the wild-type placenta [21].

3.4. PPARs in placental inflammatory response and in the parturition signalling

At this stage of our knowledge of PPARs, the most interesting results have been obtained with the study of their involvement in the inflammation process, which may be linked to labor at term and also to the premature rupture of fetal membranes (see Figure 3). Term labor is associated with an increase in proinflammatory proteins and cytokines such as IL1β, IL6, IL8, IL10, and TNF-α. This increase in proinflammatory proteins and cytokines induces uterine contractions. PPARγ ligands have been demonstrated to inhibit the secretion of IL6, IL8, and TNF-α in amnion and chorion [31], highlighting the role of PPARs in the regulation of the inflammatory response in human gestational tissues and cells [32–35]. The parathyroid hormone-related protein (presenting a cytokine-like action) is involved in many processes during normal and pathological pregnancies, and is decreased by PPARγ stimulation [36], which also blocks proinflammatory cytokine release by adiponectin and leptin [37]. The production of prostaglandins by the endometrium, the myometrium, and the fetal membranes induces the contraction of the myometrium during labor. This generation of uterotonic prostaglandins correlates with the increased prostaglandin-endoperoxide synthase type 2/cyclooxygenase type 2 (COX-2) activity and the increased secretory phospholipase A2-IIA (sPLA2) mRNA, proteins and activities. By inhibiting the production of the COX-2 and sPLA2 in fetal membranes, PPARγ promotes the quiescence of the uterus during gestation [34]. The molecular action of 15dPGJ2 seems to involve interactions of the NF-Kappa B signaling pathway, inducing reduction of PGF2α, PGE2, and MMP9 release in the placental environment [31]. This suppressive action of PPARγ on inflammation is apparently time-dependent during pregnancy. The PPARγ level of expression remains stable throughout gestation, except for the period just before labor, when its expression in fetal membranes declines. This reduction is coincidental with a relative increase in COX-2 expression [38]. Further work has shown this simple scheme to be more complex. While the expression of PPARα does not change at term in amnion, it decreases in chorion. An increase was also demonstrated for PPARβ/δ in chorionic and amniotic zones [11]. These last two findings raise the question of the involvement of the α and β isoforms in this process. The absence of a real link between COX-2 and PPARγ is presented by Lindstrom and Bennett [39]. Finally, the PPAR action seems to be concentration-dependent. A small amount of 15dPGJ2 ($<0.1\,\mu$M) acts through the PPARγ signaling pathway, where at high concentration ($1\,\mu$M) its actions are most probably mediated through other pathways: PPARβ/δ and/or an

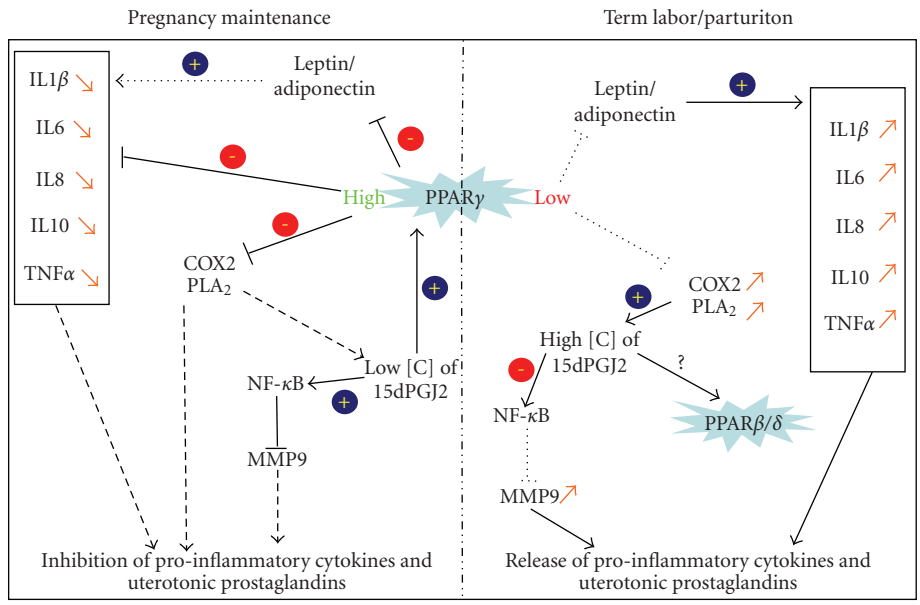


Figure 3: Schematic representation of PPARγ implication in pregnancy maintenance and labor. IL1β: Interleukin 1β; IL6: Interleukin 6; IL8: Interleukin 8; IL10: Interleukin 10, TNFα: Tumor Necrosis Factor α; COX2: Cyclo-oxygenase type 2; PLA_2: Phospholipase A2; NF-κB: Nuclear Factor-Kappa B; MMP9: Matrix Metalloproteinase 9; 15dPGJ2: 15-Deoxy-Δ12, 14-prostaglandin J2.

inhibition of NF-κB independent of PPARs [35]. Furthermore, 15dPGJ2 and troglitazone were also demonstrated to have some antiinflammatory or apoptosis-induction specific effects by PPARγ-independent pathways. This was suggested by the work of Lappas et al. on human gestational tissues, demonstrating that this effect could passed by antagonist effect of 15dPGJ2 on the NF-κB pathways and antioxidant effects of the troglitazone, a synthetic ligand of PPARγ [31].

3.5. PPARs in placental and amniotic membranes pathologies

In contrast to the different roles described for PPARs during human placentation, only a few studies on PPARs and placental pathologies have been conducted. In choriocarcinoma and hydatiform moles, a downregulation of the PPARγ expression is observed but this real influence needs to be elucidated [40]. The potential involvement of PPARγ on preeclampsia is suggested by the fact that this pathology is associated with an increased peroxidation in trophoblasts [41, 42]. An overproduction of 15-HETE has also been noted, suggesting a deregulation of PPARγ [43]. This can cause a strong transactivation of PPARγ during early pregnancy, resulting in a reduction of extravillous trophoblastic invasion, one cellular explanation often cited in the physiopathology of preeclampsia [44, 45]. Other abnormal transactivation of PPARs may be hypothesized to explain placental pathologies. The 15dPGJ2 has been shown to induce apoptosis of the placental (JEG-3) and amniotic (WISH) established cell line, [46, 47]. An excess of 15dPGJ2 production can be a source of placental dysfunction linked to an increase in trophoblastic death. It is also established that deletion of PPARγ, PPARβ/δ, and some of their coactivators (PBP, PRIP, and RAP250) induce abnormal placental phenotypes (abruption, reduction of fetomaternal exchanges, and alterations of trophoblastic differentiation) in null mutants [21, 23, 24, 26, 48, 49]. Chromosomal and/or genetic alterations (point mutation or deletion) may occur for these genes, inducing human placental alterations. The placental 11β hydroxysteroid dehydrogenase type 2 is a target gene of PPARs [50]. This enzyme plays a key role in fetal development by controlling fetal exposure to maternal glucocorticoids. An abnormal regulation by PPARs may result in an absence of fetal protection. In the rat placental HRP-1 established cell line, the phthalate and derivatives transactivate PPARs (α and γ) induced an increase in uptake rates of fetal essential fatty acid and the transport of arachidonic and docosahexaenoic acid [51]. If such a mechanism can be induced by the phthalates during human placentation, this may strongly affect the fetal essential fatty acid content during growth.

Gestational diabetes is linked to impaired lipids metabolism [52]. Decreased 15dPGJ2 in blood of diabetic mothers is also linked to a decrease in placental PPARγ expression. The inhibition of PPARγ results in an induction of a placental proinflammatory environment associated with an increase in nitrogen monoxide production and release, which can impair fetoplacental development [53, 54].

The PPAR regulation of inflammation may be very important in another obstetrical pathology of the amniotic membranes: the chorioamnionitis. This pathology, usually due to an ascendant colonization of pathogenic microorganisms from the vagina to the uterus, is closely associated with preterm labor and premature rupture of membranes (chorion and amnion). These ruptures of membranes seem to arise from deregulated proinflammatory factor synthesis. It has already been reported in this pathology that IL1β, IL6,

IL8, TNF-α, and prostaglandinE(2) show inadequate concentrations in placental membrane and in amniotic fluid [55–58]. As PPARs may be involved in the occurrence and control of this inflammatory response, further studies are needed to assess their importance in this process and to find new possible therapeutic strategies to prevent this damaging pathology.

More generally, the use of natural and synthetic PPAR ligands looks to be a promising way in preventing placental pathologies such as endometriosis or preeclampsia. An interesting study also demonstrates that the reduction of LPS induction of cytokines is reduced by PPARγ ligands in fetal membranes. Nevertheless, the few studies already conducted were done practically only on animal (rodent) models and looks to have positive effects on the pathologies (for review see Toth et al. [59]). Till now, the major problem using, for example, TZD (thiazolidinedionzes) linking to the PPARγ pathways still the numerous adverse effects of this kind of treatment (e.g., weight gain, anemia, leukopenia, etc.). These facts and the potential placental impacts raised also the question of the use of these medical drugs to treat the gestational diabetes. Perhaps, at the level of clinician actual knowledge, PPARγ and its ligands could be used in a first time, only as good early marker candidates for the diagnosis of pregnancy pathologies like, for example, preeclampsia.

4. CONCLUSION

Since the discovery of the PPARs, there has been a marked increase in available data on their involvement in mammalian development. Concerning the placenta, all PPARs, but particularly PPARγ, are essential for multiple physiological functions of the trophoblastic and amniotic parts, leading to major involvement of PPARs in the pathophysiology of gestational diseases. However, special care must be taken when this particular PPAR signaling cascade is involved, because part of the regulation may involve PPAR ligand signalling (by the natural 15dPGJ2 ligand or the troglitazone synthetic ligand) but may be transduced by independent nuclear receptor pathways (as, e.g., by antagonizing effects on NF-κB pathway for 15dPGJ2 and by acting as an antioxidant for troglitazone). This last point introduces a new level of complexity in PPAR biology. It does not close preclusion of the eventual use of PPARs for therapeutic treatment during pregnancy, but future medical applications seem still to be a long way off. We can reasonably expect to see some obstetrical use of PPARs in diagnosis (detection of PPARs mutations in intrauterine growth retardation, predisposition of preeclampsia) and therapeutics (tocolysis or treatment of chorioamniotis).

REFERENCES

[1] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[2] L. Michalik, B. Desvergne, C. Dreyer, M. Gavillet, R. N. Laurini, and W. Wahli, "PPAR expression and function during vertebrate development," *International Journal of Developmental Biology*, vol. 46, no. 1, pp. 105–114, 2002.

[3] L. Fajas, D. Auboeuf, E. Raspe, et al., "The organization, promoter analysis, and expression of the human PPARγ gene," *The Journal of Biological Chemistry*, vol. 272, no. 30, pp. 18779–18789, 1997.

[4] T. Fournier, V. Tsatsaris, K. Handschuh, and D. Evain-Brion, "PPARs and the placenta," *Placenta*, vol. 28, no. 2-3, pp. 65–76, 2007.

[5] P. Escher and W. Wahli, "Peroxisome proliferator-activated receptors: insight into multiple cellular functions," *Mutation Research*, vol. 448, no. 2, pp. 121–138, 2000.

[6] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptors: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[7] W. T. Schaiff, Y. Barak, and Y. Sadovsky, "The pleiotropic function of PPAR γ in the placenta," *Molecular and Cellular Endocrinology*, vol. 249, no. 1-2, pp. 10–15, 2006.

[8] V. Y. Ng, Y. Huang, L. M. Reddy, J. R. Falck, E. T. Lin, and D. L. Kroetz, "Cytochrome P450 eicosanoids are activators of peroxisome proliferator-activated receptor α," *Drug Metabolism and Disposition*, vol. 35, no. 7, pp. 1126–1134, 2007.

[9] P. Escher, O. Braissant, S. Basu-Modak, L. Michalik, W. Wahli, and B. Desvergne, "Rat PPARs: quantitative analysis in adult rat tissues and regulation in fasting and refeeding," *Endocrinology*, vol. 142, no. 10, pp. 4195–4202, 2001.

[10] Q. Wang, H. Fujii, and G. T. Knipp, "Expression of PPAR and RXR isoforms in the developing rat and human term placentas," *Placenta*, vol. 23, no. 8-9, pp. 661–671, 2002.

[11] E. B. E. Berry, R. Eykholt, R. J. A. Helliwell, R. S. Gilmour, M. D. Mitchell, and K. W. Marvin, "Peroxisome proliferator-activated receptor isoform expression changes in human gestational tissues with labor at term," *Molecular Pharmacology*, vol. 64, no. 6, pp. 1586–1590, 2003.

[12] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[13] B. E. Schwarz, F. M. Schultz, P. C. Macdonald, and J. M. Johnston, "Initiation of human parturition. III. fetal membrane content of prostaglandin E2 and F2α precursor," *Obstetrics and Gynecology*, vol. 46, no. 5, pp. 564–568, 1975.

[14] J. R. Okita, P. C. MacDonald, and J. M. Johnston, "Mobilization of arachidonic acid from specific glycerophospholipids of human fetal membranes during early labor," *Journal of Biological Chemistry*, vol. 257, no. 23, pp. 14029–14034, 1982.

[15] M. D. Mitchell, D. L. Kraemer, and D. M. Strickland, "The human placenta: a major source of prostaglandin D_2," *Prostaglandins Leukotrienes and Medicine*, vol. 8, no. 4, pp. 383–387, 1982.

[16] R. J. Helliwell, J. A. Keelan, K. W. Marvin, et al., "Gestational age-dependent up-regulation of prostaglandin D synthase (PGDS) and production of PGDS-derived antiinflammatory prostaglandins in human placenta," *Journal of Clinical Endocrinology & Metabolism*, vol. 91, no. 2, pp. 597–606, 2006.

[17] L. L. Waite, E. C. Person, Y. Zhou, K. H. Lim, T. S. Scanlan, and R. N. Taylor, "Placental peroxisome proliferator-activated receptor-γ is up-regulated by pregnancy serum," *Journal of Clinical Endocrinology & Metabolism*, vol. 85, no. 10, pp. 3808–3814, 2000.

[18] H. Li, X. Z. Ruan, S. H. Powis, et al., "EPA and DHA reduce LPS-induced inflammation responses in HK-2 cells: evidence for a PPAR-γ-dependent mechanism," *Kidney International*, vol. 67, no. 3, pp. 867–874, 2005.

[19] A. De Paulis, A. Ciccarelli, I. Marinò, G. De Crescenzo, D. Marinò, and G. Marone, "Human synovial mast cells: II. heterogeneity of the pharmacologic effects of antiinflammatory and immunosuppressive drugs," *Arthritis and Rheumatism*, vol. 40, no. 3, pp. 469–478, 1997.

[20] H. Fahmi, J. A. Di Battista, J. P. Pelletier, F. Mineau, P. Ranger, and J. Martel-Pelletier, "Peroxisome proliferator-activated receptor γ activators inhibit interleukin-1β-induced nitric oxide and matrix metalloproteinase 13 production in human chondrocytes," *Arthritis & Rheumatism*, vol. 44, no. 3, pp. 595–607, 2001.

[21] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPAR γ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[22] V. Sapin, L. Blanchon, A. F. Serre, D. Lemery, B. Dastugue, and S. J. Ward, "Use of transgenic mice model for understanding the placentation: towards clinical applications in human obstetrical pathologies?" *Transgenic Research*, vol. 10, no. 5, pp. 377–398, 2001.

[23] P. Antonson, G. U. Schuster, L. Wang, et al., "Inactivation of the nuclear receptor coactivator RAP250 in mice results in placental vascular dysfunction," *Molecular and Cellular Biology*, vol. 23, no. 4, pp. 1260–1268, 2003.

[24] Y. Zhu, C. Qi, Y. Jia, J. S. Nye, M. S. Rao, and J. K. Reddy, "Deletion of PBP/PARBP, the gene for nuclear receptor coactivator peroxisome proliferator-activated receptor-binding protein, results in embryonic lethality," *Journal of Biological Chemistry*, vol. 275, no. 20, pp. 14779–14782, 2000.

[25] Y.-J. Zhu, S. E. Crawford, V. Stellmach, et al., "Coactivator PRIP, the peroxisome proliferator-activated receptor-interacting protein, is a modulator of placental, cardiac, hepatic, and embryonic development," *Journal of Biological Chemistry*, vol. 278, no. 3, pp. 1986–1990, 2003.

[26] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[27] W. T. Schaiff, F. F. Knapp Jr., Y. Barak, T. Biron-Shental, D. M. Nelson, and Y. Sadovsky, "Ligand-activated PPARγ alters placental morphology and placental fatty acid uptake in mice," *Endocrinology*, vol. 148, no. 8, pp. 3625–3634, 2007.

[28] W. T. Schaiff, I. Bildirici, M. Cheong, P. L. Chern, D. M. Nelson, and Y. Sadovsky, "Peroxisome proliferator-activated receptor-γ and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 7, pp. 4267–4275, 2005.

[29] I. Bildirici, C.-R. Roh, W. T. Schaiff, B. M. Lewkowski, D. M. Nelson, and Y. Sadovsky, "The lipid droplet-associated protein adipophilin is expressed in human trophoblasts and is regulated by peroxisomal proliferator-activated receptor-γ/Retinoid X Receptor," *Journal of Clinical Endocrinology and Metabolism*, vol. 88, no. 12, pp. 6056–6062, 2003.

[30] A. K. Duttaroy, "Fetal growth and development: roles of fatty acid transport proteins and nuclear transcription factors in human placenta," *Indian Journal of Experimental Biology*, vol. 42, no. 8, pp. 747–757, 2004.

[31] M. Lappas, K. Yee, M. Permezel, and G. E. Rice, "Lipopolysaccharide and TNF-α activate the nuclear factor kappa B pathway in the human placental JEG-3 cells," *Placenta*, vol. 27, no. 6-7, pp. 568–575, 2006.

[32] D. A. Kniss, "Cyclooxygenases in reproductive medicine and biology," *Journal of the Society for Gynecologic Investigation*, vol. 6, no. 6, pp. 285–292, 1999.

[33] M. Lappas, M. Permezel, H. M. Georgiou, and G. E. Rice, "Regulation of proinflammatory cytokines in human gestational tissues by peroxisome proliferator-activated receptor-γ: effect of 15-deoxy-$\Delta^{12,14}$-PGJ_2 and troglitazone," *Journal of Clinical Endocrinology & Metabolism*, vol. 87, no. 10, pp. 4667–4672, 2002.

[34] W. E. Ackerman, X. L. Zhang, B. H. Rovin, and D. A. Kniss, "Modulation of cytokine-induced cyclooxygenase 2 expression by PPARG ligands through NFκB signal disruption in human WISH and amnion cells," *Biology of reproduction*, vol. 73, no. 3, pp. 527–535, 2005.

[35] E. B. Berry, J. A. Keelan, R. J. Helliwell, R. S. Gilmour, and M. D. Mitchell, "Nanomolar and micromolar effects of 15-deoxy- $\Delta^{12,14}$-prostaglandin J_2 on amnion-derived wish epithelial cells: differential roles of peroxisome proliferator-activated receptors γ and δ and nuclear factor κB," *Molecular Pharmacology*, vol. 68, no. 1, pp. 169–178, 2005.

[36] M. Lappas and G. E. Rice, "Phospholipase A_2 isozymes in pregnancy and parturition," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 70, no. 2, pp. 87–100, 2004.

[37] M. Lappas, M. Permezel, and G. E. Rice, "Leptin and adiponectin stimulate the release of proinflammatory cytokines and prostaglandins from human placenta and maternal adipose tissue via nuclear factor-κB, peroxisomal proliferator-activated receptor-γ and extracellularly regulated kinase 1/2," *Endocrinology*, vol. 146, no. 8, pp. 3334–3342, 2005.

[38] L. R. Dunn-Albanese, W. E. Ackerman, Y. Xie, J. D. Iams, and D. A. Kniss, "Reciprocal expression of peroxisome proliferator-activated receptor-γ and cyclooxygenase-2 in human term parturition," *American Journal of Obstetrics and Gynecology*, vol. 190, no. 3, pp. 809–816, 2004.

[39] T. M. Lindstrom and P. R. Bennett, "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 inhibits interleukin-1β-induced nuclear factor-κb in human amnion and myometrial cells: mechanisms and implications," *Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 6, pp. 3534–3543, 2005.

[40] L. Capparuccia, D. Marzioni, A. Giordano, et al., "PPARγ expression in normal human placenta, hydatidiform mole and choriocarcinoma," *Molecular Human Reproduction*, vol. 8, no. 6, pp. 574–579, 2002.

[41] J. M. Roberts, R. N. Taylor, T. J. Musci, G. M. Rodgers, C. A. Hubel, and M. K. McLaughlin, "Preeclampsia: an endothelial cell disorder," *American Journal of Obstetrics and Gynecology*, vol. 161, no. 5, pp. 1200–1204, 1989.

[42] D. Ware Branch, M. D. Mitchell, E. Miller, W. Palinski, and J. L. Witztum, "Pre-eclampsia and serum antibodies to oxidised low-density lipoprotein," *Lancet*, vol. 343, no. 8898, pp. 645–646, 1994.

[43] R. D. Johnson, K. L. Polakoski, X. Huang, Y. Sadovsky, and D. M. Nelson, "The release of 15-hydroxyeicosatetraenoic acid by human placental trophoblast is increased in preeclampsia," *American Journal of Obstetrics and Gynecology*, vol. 178, no. 1 part 1, pp. 54–58, 1998.

[44] R. L. Schild, W. T. Schaiff, M. G. Carlson, E. J. Cronbach, D. M. Nelson, and Y. Sadovsky, "The activity of PPAR γ in primary human trophoblasts is enhanced by oxidized lipids," *Journal of Clinical Endocrinology and Metabolism*, vol. 87, no. 3, pp. 1105–1110, 2002.

[45] T. Fournier, L. Pavan, A. Tarrade, et al., "The role of PPAR-γ/RXR-α heterodimers in the regulation of human trophoblast invasion," *Annals of the New York Academy of Sciences*, vol. 973, pp. 26–30, 2002.

[46] J. A. Keelan, T. A. Sato, K. W. Marvin, J. Lander, R. S. Gilmour, and M. D. Mitchell, "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2, a ligand for peroxisome proliferator-activated receptor-γ, induces apoptosis in JEG3 choriocarcinoma cells," *Biochemical and Biophysical Research Communications*, vol. 262, no. 3, pp. 579–585, 1999.

[47] J. Keelan, R. Helliwell, B. Nijmeijer, et al., "15-$\Delta^{12,14}$-prostaglandin J_2-induced apoptosis in amnion-like WISH cells," *Prostaglandins and Other Lipid Mediators*, vol. 66, no. 4, pp. 265–282, 2001.

[48] S.-Q. Kuang, L. Liao, H. Zhang, et al., "Deletion of the cancer-amplified coactivator AIB3 results in defective placentation and embryonic lethality," *Journal of Biological Chemistry*, vol. 277, no. 47, pp. 45356–45360, 2002.

[49] K. Nadra, S. I. Anghel, E. Joye, et al., "Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor β/δ," *Molecular and Cellular Biology*, vol. 26, no. 8, pp. 3266–3281, 2006.

[50] L. Julan, H. Guan, J. P. Van Beek, and K. Yang, "Peroxisome proliferator-activated receptor δ suppresses 11β-hydroxysteroid dehydrogenase type 2 gene expression in human placental trophoblast cells," *Endocrinology*, vol. 146, no. 3, pp. 1482–1490, 2005.

[51] Y. Xu, T. J. Cook, and G. T. Knipp, "Effects of di-(2-ethylhexyl)-phthalate (DEHP) and its metabolites on fatty acid homeostasis regulating proteins in rat placental HRP-1 trophoblast cells," *Toxicological Sciences*, vol. 84, no. 2, pp. 287–300, 2005.

[52] E. Capobianco, A. Jawerbaum, V. White, C. Pustovrh, D. Sinner, and E. T. Gonzalez, "Elevated levels of endothelin-1 and prostaglandin E_2 and their effect on nitric oxide generation in placental tissue from neonatal streptozotocin-induced diabetic rats," *Prostaglandins Leukotrienes and Essential Fatty Acids*, vol. 68, no. 3, pp. 225–231, 2003.

[53] A. Jawerbaum, E. Capobianco, C. Pustovrh, et al., "Influence of peroxisome proliferator-activated receptor γ activation by its endogenous ligand 15-deoxy $\Delta^{12,14}$ prostaglandin J_2 on nitric oxide production in term placental tissues from diabetic women," *Molecular Human Reproduction*, vol. 10, no. 9, pp. 671–676, 2004.

[54] A. Jawerbaum and E. González, "Diabetic pregnancies: the challenge of developing in a pro-inflammatory environment," *Current Medicinal Chemistry*, vol. 13, no. 18, pp. 2127–2138, 2006.

[55] M. J. Willi, M. Winkler, D.-C. Fischer, T. Reineke, H. Maul, and W. Rath, "Chorioamnionitis: elevated interleukin-6 and interleukin-8 concentrations in the lower uterine segment," *Journal of Perinatal Medicine*, vol. 30, no. 4, pp. 292–296, 2002.

[56] V. Zaga, G. Estrada-Gutierrez, J. Beltran-Montoya, R. Maida-Claros, R. Lopez-Vancell, and F. Vadillo-Ortega, "Secretions of interleukin-1β and tumor necrosis factor α by whole fetal membranes depend on initial interactions of amnion or chorio-odecidua with lipopolysaccharides or group B streptococci," *Biology of Reproduction*, vol. 71, no. 4, pp. 1296–1302, 2004.

[57] B. Jacobsson, I. Mattsby-Baltzer, and H. Hagberg, "Interleukin-6 and interleukin-8 in cervical and amniotic fluid: relationship to microbial invasion of the chorioamniotic membranes," *Journal of Obstetrics and Gynaecology*, vol. 112, no. 6, pp. 719–724, 2005.

[58] V. Zaga-Clavellina, G. G. Lopez, G. Estrada-Gutierrez, et al., "Incubation of human chorioamniotic membranes with Candida albicans induces differential synthesis and secretion of interleukin-1β, interleukin-6, prostaglandin E_2, and 92 kDa type IV collagenase," *Mycoses*, vol. 49, no. 1, pp. 6–13, 2006.

[59] B. Toth, D. Hornung, C. Scholz, S. Djalali, K. Friese, and U. Jeschke, "Peroxisome proliferator-activated receptors: new players in the field of reproduction," *American Journal of Reproductive Immunology*, vol. 58, no. 3, pp. 289–310, 2007.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 459030, 8 pages
doi:10.1155/2008/459030

Review Article

The Roles of PPARs in the Fetal Origins of Metabolic Health and Disease

William D. Rees,[1] Christopher J. McNeil,[1] and Christopher A. Maloney[1, 2]

[1] *The Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, Scotland*
[2] *Human Nutrition Unit, School of Molecular and Microbial Biosciences, The University of Sydney, NSW 2006, Australia*

Correspondence should be addressed to William D. Rees, wdr@rri.sari.ac.uk

Received 12 June 2007; Accepted 29 August 2007

Recommended by P. Froment

Beyond the short-term effects on fertility, there is increasing evidence that obesity or the consumption of an inappropriate diet by the mother during pregnancy adversely affects the long-term health of her offspring. PPAR and RXR isotypes are widely expressed in reproductive tissues and in the developing fetus. Through their interactions with fatty acids, they may mediate adaptive responses to the changes in the maternal diet. In the maturing follicle, PPAR-γ has an important role in the granulosa cells that surround the maturing oocyte. After fertilisation, PPAR-γ and PPAR-β/δ are essential regulators of placentation and the subsequent development of key metabolic tissues such as skeletal muscle and adipose cells. Activation of PPAR-γ and PPAR-β/δ during fetal development has the potential to modify the growth and development of these tissues. PPAR-α is expressed at low levels in the fetal liver, however, this expression may be important, as changes in the methylation of DNA in its promoter region are reported to take place during this period of development. This epigenetic modification then programmes subsequent expression. These findings suggest that two separate PPAR-dependent mechanisms may be involved in the fetal adaptations to the maternal diet, one, mediated by PPAR-γ and PPAR-β/δ, regulating cell growth and differentiation; and another adapting long-term lipid metabolism via epigenetic changes in PPAR-α to optimise postnatal survival.

1. INTRODUCTION

Human diets in the developed world have changed dramatically during the last century. An increase in the consumption of fat, coupled with a fall in physical activity, has led to unprecedented rates of obesity in Western populations. However, the complications associated with these changes in lifestyle extend beyond the present generation and threaten the next one. There is an overwhelming body of evidence showing that the diet and body composition of the mother modifies the risk of the offspring developing cardiovascular and metabolic diseases later in life [1]. Increased body weight and decreased physical activity are also associated with ovulatory dysfunction and reduced fertility [2, 3]. As the primary regulators of lipid metabolism at the cellular level, the peroxisome proliferator-activated receptor (PPAR) isotypes help to maintain metabolic homeostasis when the energy or lipid composition of the diet changes. The PPARs are widely expressed in the reproductive tissues and in the developing fetus, where by analogy with their function in adult tissues, they may mediate adaptations to the nutrient supply during reproduction. Recent studies of the mechanisms of metabolic programming have begun to shed light on the involvement of the PPARs in the fetal origins of health and disease [4–6]. In this review, we will consider the possible roles of PPAR isotypes and the related retinoid X receptor isotypes (RXR) in the developmental adaptations that occur in response to fluctuations in the maternal diet.

2. THE ROLE OF LIPID METABOLISM IN THE FETAL ORIGINS OF DISEASE

Much of the evidence from human and animal studies suggests that inappropriate energy metabolism during pregnancy has an adverse effect on fetal development and is an important factor in metabolic programming. In human populations, birth weight data is frequently used as a surrogate measure of fetal growth and hence the nutrient supply.

Several studies have shown that there is a strong relationship between weight at birth and the risk of impaired glucose tolerance in adult life [7] and that there is a U-shaped relationship between birth weight and obesity in adult life [8]. Rapid catch-up growth in infancy following a period of fetal growth restriction carries the highest risk of central obesity in adulthood, particularly in babies that are thin at birth and small for gestational age. Importantly it is thinness at birth and not birth weight itself that explains the relationship between low birth weight and the long-term metabolic complications, suggesting that changes in the development of adiposity during fetal life is a critical factor [9]. At the other end of the spectrum, there is a positive association between birth weight and body mass index at age 20, suggesting that elevated birth weight is also associated with an increase in adiposity [10]. Mothers who are diabetic or develop serious gestational diabetes give birth to babies that are large for gestational age. These offspring of hyperglycaemic mothers have a much higher risk of developing metabolic syndrome in childhood, demonstrating a link between maternal blood glucose levels and perturbed metabolism in the offspring [11]. Thus, it appears that there are two different mechanisms underlying the development of glucose intolerance and obesity in adult life: one at the higher end of the birth weight spectrum, associated with maternal hyperglycemia, and another at the lower end associated with the development of adipose tissue [8].

Animal models for fetal programming also implicate lipid and carbohydrate metabolism in the programming process. Pertinent to this discussion of the role of PPARs in development are studies in which the maternal diet modifies lipid metabolism. Feeding rats a high-fat diet during gestation programmes glucose intolerance, pancreatic beta-cell dysfunction, and increases the body weight of their offspring [12, 13]. Other metabolic perturbations in gestation such as modest protein restriction, or iron deficiency also lead to persistent changes in the offspring. These also are linked indirectly to changes in lipid metabolism in the dam. In the case of protein restriction, triglyceride concentrations in the maternal plasma are increased in animals fed the low-protein rations and this is associated with changes in the expression of PPAR-α in the offspring [14]. This increase in plasma triglycerides can be modulated by the fatty acid composition of the diet [15], an intervention which also modifies the effects of protein deficiency on glucose tolerance in the offspring [16]. Micronutrients in the maternal diet are also important and there is evidence that their effects are also mediated indirectly through changes in lipid metabolism. For example, iron deficiency reduces triglyceride concentrations in the liver of the Fe-restricted fetuses by approximately 25% with corresponding changes in the expression of SREBP-1c and its downstream genes [17]. There are also reports that vitamin A deficiency during gestation is associated with impaired glucose tolerance in adult life [18].

Both human and animal studies suggest that there are a number of critical windows in development where changes in the maternal diet can influence the long-term outcome of the offspring. These span the entire reproductive cycle from the preconception period when the germ cells mature right through gestation and into the lactation period (Figure 1).

3. PPARs DURING PRECONCEPTION DEVELOPMENT

Evolutionary forces favour animals able to regulate their fertility in response to the availability of nutrients in the environment. Metabolic status at the start of the reproductive cycle before conception is a good guide to subsequent success. Whilst these controls have developed to deal with famine, inappropriate responses to dietary excess or imbalance are more of concern in the modern world. Because of the links between body composition and infertility, there is considerable interest in the mechanisms by which nutrient sensors, such as the PPARs, regulate the maturation of the oocyte.

All of the PPAR isotypes are expressed in the rat ovary. PPAR-γ is found in the granulosa cells that surround and support the maturing oocyte. PPAR-α and PPAR-β/δ are present at lower levels in the thecal and stromal cells [19]. The low levels of the PPAR-α and PPAR-β/δ isotypes suggest that they play a role in basal ovarian function whereas the higher levels of the PPAR-γ isotype imply a more specific function in the granulosa cell [20]. However, PPAR-γ is not essential, as mice with a targeted deletion of the gene in granulosa cells are able to reproduce successfully, albeit with reduced fertility, related to a reduced implantation rate [21]. Instead PPAR-γ appears to be a negative regulator of follicular growth and differentiation. The viability of rat granulosa cells is reduced when they are treated with a specific PPAR-γ agonist, suggesting that PPAR-γ activation suppresses follicle development [22]. Recent studies also suggest that follicular functions are sensitive to dietary factors in vivo. Trans fatty acids increase the risk of ovulatory infertility when they replace the unsaturated fats that are commonly found in vegetable oils [23]. Since these fatty acids are able to activate PPAR-γ, the data suggest that it may be an important transducer.

Effects on ovulation, mediated by PPAR-γ in conjunction with RXR isotypes, may go beyond effects on fertility. Early embryonic development is dependent on stores of maternally derived factors passed from the granulosa cells to the oocyte during maturation. If these stores are depleted due to poor granulosa cell function, there may be an effect on the immediate postnatal development following fertilisation. A small change in growth during this early stage may be the start of a chain of events leading to long-lasting effects, such as elevated blood pressure in the offspring [24].

The PPARs are also expressed in the testis [20] where lipid metabolism and especially the β-oxidation of fatty acids are important for testicular function. Peroxisome proliferators, such as phthalates are known testicular toxicants. They interfere with the transcriptional activity of RAR-α in Sertoli cells by increasing the nuclear localisation of PPAR-α and increasing its transcriptional activity [25]. The extensive accumulation of neutral lipids in the testis has been observed in a number of mouse models in which key genes such as RXR-β have been deleted [26]. These finding suggest that the regulation of lipid metabolism by PPAR and RXR may be important in the regulation of male fertility. Unlike ovulation, the

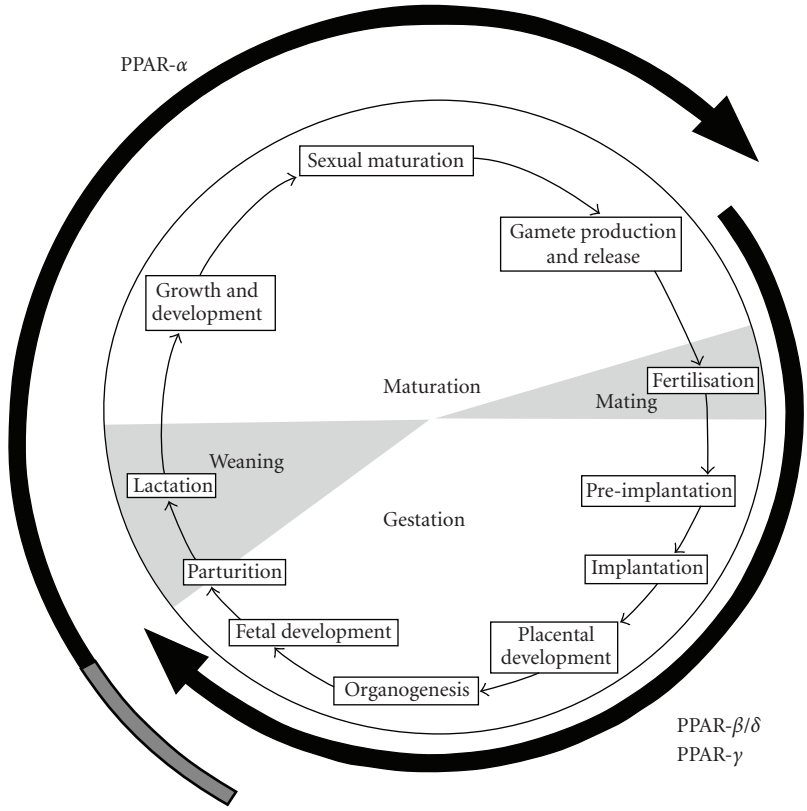


Figure 1: PPAR isotype expression and programming during the reproductive cycle. The PPAR-β/δ and PPAR-γ isotypes regulate the growth of key organs and manage the development of adipose tissue during fetal development. During the later stages of fetal development epigenetic programming of PPAR-α (represented by the grey section of the arrow) programmes long-term postnatal regulation of energy metabolism.

impact of high-fat diets and obesity on the function of PPARs during spermatogenesis is a relatively unexplored area. It is interesting to note that there has been a marked decrease in male fertility concomitant with the developing obesity epidemic suggesting that this is an area in need of further study.

4. PPARs DURING IMPLANTATION AND PLACENTATION

Following fertilisation there is a rapid differentiation of the early embryo into specialised cell types. This is the first stage of cellular differentiation when the tissues within the embryo begin to develop specialised metabolic functions. With this evolving complexity there is a requirement for mechanisms to maintain metabolic homeostasis between the different tissues. As the interface with the maternal circulation, the extraembryonic endoderm and then the placenta perform vital functions in regulating the nutrient supply to the developing tissues. The growth of the fetus is dependent on appropriate placental development, as a small placenta will restrict the availability of nutrients.

The PPAR isotypes play an important role in regulating the implantation of the embryo and the development of the placenta [27]. The mRNAs for RXR-α, RXR-β and PPAR-γ as well as the RXR-β and PPAR-γ proteins, have been detected in the trophectoderm and inner cell mass cells of intact and hatched blastocysts [28]. In mice, nutrients are transported through this extraembryonic endoderm prior to implantation. In cultures of trophoblast cells, activation of PPAR-γ or RXR with selective agonists enhances the uptake of free fatty acids and increases the accumulation of neutral lipids by increasing the expression of the FATP-4 transporter located in the brush-border membrane [29]. Thus, at this very early stage of development before the placenta is fully developed, the availability of substrates can modify the use of fatty acids by the embryo. At present, little is known about the impact of high-fat diets or obesity in this period and it remains to be seen if an increased utilisation of fatty acids at this stage has any long-term impact on the fetus.

The PPAR-β/δ and PPAR-γ isotypes also regulate fatty acid metabolism after the embryo has implanted and the placenta has developed. Fatty acids are used by the developing fetus for energy metabolism, membrane biosynthesis, and synthesis of signalling molecules. The PPAR-β/δ mRNA is ubiquitously expressed throughout the placenta including the labyrinth, the spongiotrophoblast, and the giant cells. Homozygous disruption of PPAR-β/δ results in the death of the majority of fetuses between days 9.5 and 10.5 of gestation. Pathological changes are mainly found in the giant cell layer of the placenta. The time of death corresponds to the period when PPAR-β/δ controls the differentiation and accumulation of lipid droplets in these cells [30]. In contrast,

PPAR-γ is required for the development of the labyrinth layer of the placenta. The placentae of PPAR-γ null mice have impaired vascularisation [31] and fewer lipid droplets in the labyrinthine trophoblasts [32], resulting in embryonic lethality at about day 9.5 of gestation. Conversely, the activation of PPAR-γ by the administration of specific agonists in vivo reduces the thickness of the spongiotrophoblast layer, modifies the labyrinthine vasculature, and enhances fatty acid uptake and the expression of fatty acid transport proteins [33]. However, information on the action of nutritional factors is sparse. Metabolic perturbations such as those produced by experimental diabetes increase the expression of PPAR-γ and proteins that are regulated by it such as vascular endothelial growth factor [34]. These findings suggest that the PPAR-γ pathway might be involved in the impairment of placental development induced by high-glucose conditions. They also suggest that high-fat diets or obesity may also modify PPAR-γ signalling in the placenta due to high concentrations of lipids in the maternal circulation.

5. THE DEVELOPMENT OF ORGAN SYSTEMS

Further metabolic specialisation occurs within the fetus as the different organ systems develop. In the adult, the PPAR isotypes and isoforms play central roles in the metabolic interplay that occurs between the different organs. In the adult, adipose tissue, skeletal muscle, the liver, and pancreatic beta-cells are all involved in the regulation of glucose and lipid metabolism. The maternal diet has the potential to programme subsequent metabolism by modifying the development of these tissues during fetal development.

The association between thinness at birth and adult disease has been linked to the development of adipose tissue in utero, a process that involves both PPAR-γ and PPAR-β/δ. Animal studies suggest that the maternal diet does not influence either the proliferation or differentiation of preadipocyte cells in vitro [35]. Once preadipocytes have been isolated from the offspring, they proliferate and differentiate normally, suggesting that regulation must occur during fetal development. Many different transcription factors are involved in the commitment of mesenchymal stem cells to the adipocyte lineage [36]. Amongst these are PPAR-β/δ, which is expressed during the preadipose stages, and PPAR-γ, which is expressed as part of the mature adipocyte phenotype. Targeted deletions of the PPAR-β/δ and PPAR-γ genes in mice have demonstrated that both genes are essential for adipogenesis. The small numbers of PPAR-β/δ null mice that do not succumb to placental failure have an extremely lean phenotype, typified by a 2.5-fold reduction of abdominal fat mass compared with control littermates [37]. Similarly, PPAR-γ null mice, rescued by forming chimeras in which the placenta is formed from wild-type cells, die soon after birth because they are devoid of adipose tissue [32]. PPAR-γ-mediated signalling regulates adipogenesis in the adult by forming a positive feedback loop, sensitive to long-chain, saturated, and polyunsaturated fatty acids in the diet [38]. It is probable that this same system is able to regulate the development of fetal preadipose cells and adipocytes in situations where there are elevated levels of fatty acids supplied to the fetal tissues from either the maternal diet or through the mobilisation of maternal adipose reserves.

Altered muscle development may be an important element in prenatal programming of the metabolic syndrome. Skeletal muscles are a major site of carbohydrate and fatty acid metabolism and small changes induced during development have long-lasting effects. The offspring of rats fed high-energy diets (cafeteria diet) during gestation and lactation have fewer muscle fibres and more intramuscular fat, related to an increase in the expression of PPAR-γ mRNA in the muscle [39]. There is good evidence showing that both PPAR-β/δ and PPAR-γ regulate the expression of the genes involved myogenesis. Targeted expression of an activated form of PPAR-β/δ in the skeletal muscles of mice makes the animals resistant to obesity by increasing the numbers of oxidative muscle fibres [40], while the selective ablation of PPAR-β/δ induces obesity by reducing the oxidative capacity of the muscles [41]. In muscle cell cultures, PPAR-β/δ has been shown to regulate the expression of genes involved in fatty acid transport, beta-oxidation, and mitochondrial respiration [42]. Muscle specific ablation of the PPAR-γ gene in mice also produces animals that are obese and insulin resistant [43]. In contrast to the positive effects of PPAR-β/δ on myogeneisis, the overexpression of PPAR-γ in myoblast cultures has been shown to inhibit the formation of myotubes by suppressing the expression of muscle-specific myogenic proteins including myogenin, MyoD, and creatine kinase [44]. As a great deal of myogenesis takes place before birth, both PPAR-β/δ and PPAR-γ could be important regulators of fetal muscle development in response to lipids in the maternal diet.

Change in the size of the pancreatic islets due to an increase in beta-cells is an important feature of some animal models of fetal programming. PPAR-γ mediated signalling has been implicated in the regulation of beta-cell proliferation in adults. Mice in which the expression of the PPAR-γ gene was eliminated in beta-cells were found to have significant islet hyperplasia [45]. Paradoxically PPAR-γ agonists also enhance pancreatic growth [46] and the expression of key transcriptional activators required for beta-cell differentiation in cell cultures [47]. The reasons for these differences are unexplained. There is good evidence showing that changes in beta-cell expansion during the later stages of fetal development depend on glucocorticoids [48]. Thus, the role of PPAR-γ in the fetal pancreas remains unclear. However, the possibility remains that it may be important when the developing pancreas is exposed to high levels of fat from maternal obesity or high-fat diets.

The liver is the main site of PPAR-α expression in the adult, with much lower levels of the PPAR-β/δ and PPAR-γ isotypes found in this tissue. Homozygous disruption of the PPAR-α, PPAR-β/δ, and PPAR-γ genes has no effect on the development of the liver; and the offspring exhibit no apparent abnormalities [49]. However, PPAR-α is expressed in the fetal liver albeit at much lower levels than in the adult [50]; and as discussed below this fetal expression may be important in the programming of postnatal expression.

The RXR isotypes also plays a central role in organogenesis [51]. Recent studies of the mouse epidermis have suggested that 9-cis retinoic acid is not the in vivo ligand of RXR [52]. The actions of various pharmacological agents and the observation that keratinocytes do not contain retinoids suggest that fatty acids are the natural RXR ligand and that RXR is acting as a lipid sensor. Thus, it is possible that the same fatty acids are able to activate both partners of a PPAR:RXR heterodimer. If these findings hold for PPAR:RXR heterodimers in other tissues then this represents a clear mechanism by which the availability of fatty acids can influence fetal development.

6. THE PROGRAMMING OF PPAR-α EXPRESSION

Persistent alterations to the phenotype of the offspring imply stable changes in gene expression. Candidate genes for such effects arise from studies showing altered gene expression in the offspring of laboratory animals fed restricted diets. There is accumulating evidence that there are long-term changes in the stable expression of PPAR-α [14] and of genes regulated by it, including acetyl-CoA carboxylase and fatty acid synthase [16, 53]. A change in the expression of these genes is associated with impaired lipid homeostasis in the adult. Recent studies have found evidence for epigenetic changes in the PPAR-α gene which may account for this programming [4]. Analysis of genomic DNA using methylation specific restriction enzymes suggests that the methylation of the exon 1 promoter was approximately 20% lower in the offspring of rats fed a low-protein diet in gestation. At the same time, there was a 10-fold increase in the mRNA for PPAR-α. These changes were specific for PPAR-α as there was no change in the methylation status of the PPAR-γ gene. Similar epigenetic changes induced during fetal development and persisting into adult life with long-lasting effects on the physiological mechanisms have been demonstrated with the glucocorticoid receptor [54].

Nutrient sensitive transcriptional activators, such as the PPAR-α, are able to determine local chromatin structure through interactions with coactivator proteins. Indeed, these interactions are an essential component of the mechanism of transcriptional activation [55]. Even when there is no ligand present, PPARs form heterodimers with RXRα which bind to DNA in association with a number of corepressor proteins. Binding of a ligand to a PPAR dissociates the corepressor protein complex, releasing the PPAR:RXR heterodimer which then sequentially associates with various transcriptional coactivator proteins. This protein complex modifies histone and chromatin structure, making the DNA accessible for transcription while at the same time recruiting RNA polymerase II and activating the transcriptional machinery. The proteins involved in the coactivator complex include PGC-1 histone acetyl transferases, histone deacetylases and methyl transferases [55]. At present, there are no reports of coactivators with transcriptional functions specific to the PPAR subfamily. Individual coactivators are shared by many transcription factors and are involved in numerous signalling pathways [56, 57]. For example, the nuclear receptor coactivator PBP (PPAR-binding protein) functions as a coactivator for other members of the nuclear receptor family. A targeted deletion of the PBP gene in hepatocytes reduces the association of other unrelated cofactors, especially the cyclic-AMP responsive element binding protein and thyroid hormone receptor-associated proteins to the PPAR-α dependent mouse enoyl-CoA hydratase/L-3-hydroxyacyl-CoA dehydrogenase gene promoter [58]. Within the nuclear receptor coactivation complex there are some proteins, which do not directly bind to nuclear receptors but are present in the complex due to their binding to other coactivators. Amongst these are proteins that can methylate histones. It has also been suggested that changes in the recruitment of the Dnmt-1 methyl transferase to the promoter during development may be responsible for the modification of DNA methylation at the glucocorticoid receptor [59].

Thus, interactions between PPAR-α and its ligands in the liver during fetal development may be important in adapting chromatin structure, and hence long-term expression, to the nutrient supply likely to be encountered by the fetus in postnatal life. Because these modifications occur before PPAR-α is required for metabolic regulation, this may be a molecular mechanism which establishes the sensitivity of the developing tissue to nutrient signals. These modifications to the metabolic phenotype may be beneficial when nutrients are limited, as it provides a mechanism that will adapt the response of the offspring to a poor diet in the postnatal environment. Equally, when the diet is high in fat and carbohydrates, hepatic metabolism will be well adapted to direct excess fat towards storage in adipose tissue and prevent some of the adverse effects of lipotoxicity.

7. CONCLUDING REMARKS

PPAR and RXR isotypes have an essential role in the homeostatic mechanisms that maintain energy metabolism in the adult. There is now increasing evidence that they ensure that the metabolic tissues of the fetus develop in a controlled way during gestation. It appears that there may be two different PPAR-mediated mechanisms involved in the fetal origins of health and disease. One is mediated via PPAR-γ, which regulates the growth of key organs and manages the development of adipose tissue during fetal development. The other is mediated via PPAR-α in which epigenetic control preprogrammes long-term regulation of energy metabolism.

Bioactive factors such as lipids, carbohydrates, amino acids, as well as lipid-derived hormones crossing the placental barrier may disrupt this careful balance in metabolism. Critically, regulatory systems that have evolved to deal with famine are poorly suited to deal with nutrient excess. High levels of lipid, either from the diet or derived from excessive maternal stores may overwhelm the protective mechanisms offered by the PPAR receptors. Once inappropriate control points are established, then metabolic balance will be disturbed for the remainder of life. Insulin resistance programmed at fetal stages will become more pronounced with age, ultimately leading to the development of metabolic disease.

ACKNOWLEDGMENTS

Work in the authors' laboratory was supported by the Scottish Executive as part of the core funds of the Rowett Research Institute. Christopher McNeil is supported by the European Union sixth Framework programme EARNEST (CT-2005-007036). Christopher Maloney is supported by a cooperative agreement from the NIH (U01 HD044638) as a component of the NICHD Cooperative Program on Female Health and Egg Quality.

REFERENCES

[1] P. D. Gluckman and M. A. Hanson, "Living with the past: evolution, development, and patterns of disease," *Science*, vol. 305, no. 5691, pp. 1733–1736, 2004.

[2] J. W. Rich-Edwards, M. B. Goldman, W. C. Willett, et al., "Adolescent body mass index and infertility caused by ovulatory disorder," *American Journal of Obstetrics and Gynecology*, vol. 171, no. 1, pp. 171–177, 1994.

[3] J. W. Rich-Edwards, D. Spiegelman, M. Garland, et al., "Physical activity, body mass index, and ovulatory disorder infertility," *Epidemiology*, vol. 13, no. 2, pp. 184–190, 2002.

[4] K. A. Lillycrop, E. S. Phillips, A. A. Jackson, M. A. Hanson, and G. C. Burdge, "Dietary protein restriction of pregnant rats induces and folic acid supplementation prevents epigenetic modification of hepatic gene expression in the offspring," *The Journal of Nutrition*, vol. 135, no. 6, pp. 1382–1386, 2005.

[5] A. Erhuma, A. M. Salter, D. V. Sculley, S. C. Langley-Evans, and A. J. Bennett, "Prenatal exposure to a low-protein diet programs disordered regulation of lipid metabolism in the aging rat," *American Journal of Physiology*, vol. 292, no. 6, pp. E1702–E1714, 2007.

[6] K. M. Godfrey, K. A. Lillycrop, G. C. Burdge, P. D. Gluckman, and M. A. Hanson, "Epigenetic mechanisms and the mismatch concept of the developmental origins of health and disease," *Pediatric Research*, vol. 61, no. 5, part 2, pp. 5R–10R, 2007.

[7] C. N. Hales and D. J. P. Barker, "Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis," *Diabetologia*, vol. 35, no. 7, pp. 595–601, 1992.

[8] E. Oken and M. W. Gillman, "Fetal origins of obesity," *Obesity Research*, vol. 11, no. 4, pp. 496–506, 2003.

[9] D. Jaquet, S. Deghmoun, D. Chevenne, D. Collin, P. Czernichow, and C. Lévy-Marchal, "Dynamic change in adiposity from fetal to postnatal life is involved in the metabolic syndrome associated with reduced fetal growth," *Diabetologia*, vol. 48, no. 5, pp. 849–855, 2005.

[10] H. T. Sørensen, S. Sabroe, K. J. Rothman, M. Gillman, P. Fischer, and T. I. A. Sørensen, "Relation between weight and length at birth and body mass index in young adulthood: cohort study," *British Medical Journal*, vol. 315, no. 7116, p. 1137, 1997.

[11] C. M. Boney, A. Verma, R. Tucker, and B. R. Vohr, "Metabolic syndrome in childhood: association with birth weight, maternal obesity, and gestational diabetes mellitus," *Pediatrics*, vol. 115, no. 3, pp. e290–e296, 2005.

[12] M. Srinivasan, S. D. Katewa, A. Palaniyappan, J. D. Pandya, and M. S. Patel, "Maternal high-fat diet consumption results in fetal malprogramming predisposing to the onset of metabolic syndrome-like phenotype in adulthood," *American Journal of Physiology*, vol. 291, no. 4, pp. E792–E799, 2006.

[13] P. D. Taylor, J. McConnell, I. Y. Khan, et al., "Impaired glucose homeostasis and mitochondrial abnormalities in offspring of rats fed a fat-rich diet in pregnancy," *American Journal of Physiology*, vol. 288, no. 1, pp. R134–R139, 2005.

[14] G. C. Burdge, E. S. Phillips, R. L. Dunn, A. A. Jackson, and K. A. Lillycrop, "Effect of reduced maternal protein consumption during pregnancy in the rat on plasma lipid concentrations and expression of peroxisomal proliferator-activated receptors in the liver and adipose tissue of the offspring," *Nutrition Research*, vol. 24, no. 8, pp. 639–646, 2004.

[15] C. J. McNeil, C. A. Maloney, S. M. Hay, and W. D. Rees, "Sources of dietary protein and lipid interact to modify maternal and fetal development in the pregnant rat," *Proceedings of the Nutrition Society*, vol. 66, no. 4a, p. 21A, 2007.

[16] C. A. Maloney, C. Lilley, A. Czopek, S. M. Hay, and W. D. Rees, "Interactions between protein and vegetable oils in the maternal diet determine the programming of the insulin axis in the rat," *British Journal of Nutrition*, vol. 97, no. 5, pp. 912–920, 2007.

[17] J. Zhang, R. M. Lewis, C. Wang, N. Hales, and C. D. Byrne, "Maternal dietary iron restriction modulates hepatic lipid metabolism in the fetuses," *American Journal of Physiology*, vol. 288, no. 1, pp. R104–R111, 2005.

[18] K. A. Matthews, W. B. Rhoten, H. K. Driscoll, and B. S. Chertow, "Vitamin A deficiency impairs fetal islet development and causes subsequent glucose intolerance in adult rats," *The Journal of Nutrition*, vol. 134, no. 8, pp. 1958–1963, 2004.

[19] C. M. Komar, O. Braissant, W. Wahli, and T. E. Curry Jr., "Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period," *Endocrinology*, vol. 142, no. 11, pp. 4831–4838, 2001.

[20] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[21] Y. Cui, K. Miyoshi, E. Claudio, et al., "Loss of the peroxisome proliferation-activated receptor gamma (PPARγ) does not affect mammary development and propensity for tumor formation but leads to reduced fertility," *Journal of Biological Chemistry*, vol. 277, no. 20, pp. 17830–17835, 2002.

[22] T. Lovekamp-Swan and C. L. Chaffin, "The peroxisome proliferator-activated receptor γ ligand troglitazone induces apoptosis and p53 in rat granulosa cells," *Molecular and Cellular Endocrinology*, vol. 233, no. 1-2, pp. 15–24, 2005.

[23] J. E. Chavarro, J. W. Rich-Edwards, B. A. Rosner, and W. C. Willett, "Dietary fatty acid intakes and the risk of ovulatory infertility," *American Journal of Clinical Nutrition*, vol. 85, no. 1, pp. 231–237, 2007.

[24] A. J. Watkins, D. Platt, T. Papenbrock, et al., "Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 104, no. 13, pp. 5449–5454, 2007.

[25] J. M. Dufour, M.-N. Vo, N. Bhattacharya, J. Okita, R. Okita, and K. H. Kim, "Peroxisome proliferators disrupt retinoic acid receptor alpha signaling in the testis," *Biology of Reproduction*, vol. 68, no. 4, pp. 1215–1224, 2003.

[26] P. Kastner, M. Mark, M. Leid, et al., "Abnormal spermatogenesis in RXRβ mutant mice," *Genes & Development*, vol. 10, no. 1, pp. 80–92, 1996.

[27] T. Fournier, V. Tsatsaris, K. Handschuh, and D. Evain-Brion, "PPARs and the placenta," *Placenta*, vol. 28, no. 2-3, pp. 65–76, 2007.

[28] M. Mohan, J. R. Malayer, R. D. Geisert, and G. L. Morgan, "Expression patterns of retinoid X receptors, retinaldehyde dehydrogenase, and peroxisome proliferator activated receptor gamma in bovine preattachment embryos," *Biology of Reproduction*, vol. 66, no. 3, pp. 692–700, 2002.

[29] W. T. Schaiff, I. Bildirici, M. Cheong, P. L. Chern, D. M. Nelson, and Y. Sadovsky, "Peroxisome proliferator-activated receptor-γ and retinoid X receptor signaling regulate fatty acid uptake by primary human placental trophoblasts," *The Journal of Clinical Endocrinology & Metabolism*, vol. 90, no. 7, pp. 4267–4275, 2005.

[30] K. Nadra, S. I. Anghel, E. Joye, et al., "Differentiation of trophoblast giant cells and their metabolic functions are dependent on peroxisome proliferator-activated receptor β/δ," *Molecular and Cellular Biology*, vol. 26, no. 8, pp. 3266–3281, 2006.

[31] R. Asami-Miyagishi, S. Iseki, M. Usui, K. Uchida, H. Kubo, and I. Morita, "Expression and function of PPARγ in rat placental development," *Biochemical and Biophysical Research Communications*, vol. 315, no. 2, pp. 497–501, 2004.

[32] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[33] W. T. Schaiff, F. F. Knapp Jr., Y. Barak, T. Biron-Shental, D. M. Nelson, and Y. Sadovsky, "Ligand-activated peroxisome proliferator activated receptor γ alters placental morphology and placental fatty acid uptake in mice," *Endocrinology*, vol. 148, no. 8, pp. 3625–3634, 2007.

[34] N. Suwaki, H. Masuyama, A. Masumoto, N. Takamoto, and Y. Hiramatsu, "Expression and potential role of peroxisome proliferator-activated receptor γ in the placenta of diabetic pregnancy," *Placenta*, vol. 28, no. 4, pp. 315–323, 2007.

[35] F. Bieswal, S. M. Hay, C. McKinnon, et al., "Prenatal protein restriction does not affect the proliferation and differentiation of rat preadipocytes," *The Journal of Nutrition*, vol. 134, no. 6, pp. 1493–1499, 2004.

[36] E. D. Rosen and O. A. MacDougald, "Adipocyte differentiation from the inside out," *Nature Reviews Molecular Cell Biology*, vol. 7, no. 12, pp. 885–896, 2006.

[37] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[38] L. Madsen, R. K. Petersen, and K. Kristiansen, "Regulation of adipocyte differentiation and function by polyunsaturated fatty acids," *Molecular Basis of Disease*, vol. 1740, no. 2, pp. 266–286, 2005.

[39] S. A. Bayol, B. H. Simbi, and N. C. Stickland, "A maternal cafeteria diet during gestation and lactation promotes adiposity and impairs skeletal muscle development and metabolism in rat offspring at weaning," *The Journal of Physiology*, vol. 567, no. 3, pp. 951–961, 2005.

[40] Y.-X. Wang, C.-L. Zhang, R. T. Yu, et al., "Regulation of muscle fiber type and running endurance by PPARδ," *PLoS Biology*, vol. 2, no. 10, p. e294, 2004.

[41] M. Schuler, F. Ali, C. Chambon, et al., "PGC1α expression is controlled in skeletal muscles by PPARβ, whose ablation results in fiber-type switching, obesity, and type 2 diabetes," *Cell Metabolism*, vol. 4, no. 5, pp. 407–414, 2006.

[42] T. Tanaka, J. Yamamoto, S. Iwasaki, et al., "Activation of peroxisome proliferator-activated receptor δ induces fatty acid β-oxidation in skeletal muscle and attenuates metabolic syndrome," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 26, pp. 15924–15929, 2003.

[43] A. W. Norris, L. Chen, S. J. Fisher, et al., "Muscle-specific PPARγ-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones," *Journal of Clinical Investigation*, vol. 112, no. 4, pp. 608–618, 2003.

[44] J. Singh, N. K. Verma, S. M. Kansagra, B. N. Kate, and C. S. Dey, "Altered PPARγ expression inhibits myogenic differentiation in C2C12 skeletal muscle cells," *Molecular and Cellular Biochemistry*, vol. 294, no. 1-2, pp. 163–171, 2007.

[45] E. D. Rosen, R. N. Kulkarni, P. Sarraf, et al., "Targeted elimination of peroxisome proliferator-activated receptor γ in β cells leads to abnormalities in islet mass without compromising glucose homeostasis," *Molecular and Cellular Biology*, vol. 23, no. 20, pp. 7222–7229, 2003.

[46] D. M. Jia and M. Otsuki, "Troglitazone stimulates pancreatic growth in normal rats," *Pancreas*, vol. 24, no. 3, pp. 303–312, 2002.

[47] J. A. Moibi, D. Gupta, T. L. Jetton, M. Peshavaria, R. Desai, and J. L. Leahy, "Peroxisome proliferator-activated receptor-γ regulates expression of PDX-1 and NKX6.1 in INS-1 cells," *Diabetes*, vol. 56, no. 1, pp. 88–95, 2007.

[48] E. Gesina, B. Blondeau, A. Milet, et al., "Glucocorticoid signalling affects pancreatic development through both direct and indirect effects," *Diabetologia*, vol. 49, no. 12, pp. 2939–2947, 2006.

[49] S. S. Lee, T. Pineau, J. Drago, et al., "Targeted disruption of the α isoform of the peroxisome proliferator- activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators," *Molecular and Cellular Biology*, vol. 15, no. 6, pp. 3012–3022, 1995.

[50] N. Balasubramaniyan, M. Shahid, F. J. Suchy, and M. Ananthanarayanan, "Multiple mechanisms of ontogenic regulation of nuclear receptors during rat liver development," *American Journal of Physiology*, vol. 288, no. 2, pp. G251–G260, 2005.

[51] M. Mark, N. B. Ghyselinck, and P. Chambon, "Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis," *Annual Review of Pharmacology and Toxicology*, vol. 46, pp. 451–480, 2006.

[52] C. Calléja, N. Messaddeq, B. Chapellier, et al., "Genetic and pharmacological evidence that a retinoic acid cannot be the RXR-activating ligand in mouse epidermis keratinocytes," *Genes & Development*, vol. 20, no. 11, pp. 1525–1538, 2006.

[53] C. A. Maloney, A. K. Gosby, J. L. Phuyal, G. S. Denyer, J. M. Bryson, and I. D. Caterson, "Site-specific changes in the expression of fat-partitioning genes in weanling rats exposed to a low-protein diet in utero," *Obesity Research*, vol. 11, no. 3, pp. 461–468, 2003.

[54] M. Szyf, I. C. G. Weaver, F. A. Champagne, J. Diorio, and M. J. Meaney, "Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat," *Frontiers in Neuroendocrinology*, vol. 26, no. 3-4, pp. 139–162, 2005.

[55] S. Yu and J. K. Reddy, "Transcription coactivators for peroxisome proliferator-activated receptors," *Molecular and Cell Biology of Lipids*, vol. 1771, no. 8, pp. 936–951, 2007.

[56] J. N. Feige, L. Gelman, L. Michalik, B. Desvergne, and W. Wahli, "From molecular action to physiological outputs: peroxisome proliferator-activated receptors are nuclear receptors at the crossroads of key cellular functions," *Progress in Lipid Research*, vol. 45, no. 2, pp. 120–159, 2006.

[57] M. G. Rosenfeld, V. V. Lunyak, and C. K. Glass, "Sensors and signals: a coactivator/corepressor/epigenetic code for integrating signal-dependent programs of transcriptional response," *Genes & Development*, vol. 20, no. 11, pp. 1405–1428, 2006.

[58] Y. Jia, C. Qi, P. Kashireddi, et al., "Transcription coactivator PBP, the peroxisome proliferator-activated receptor (PPAR)-binding protein, is required for PPARα-regulated gene expression in liver," *Journal of Biological Chemistry*, vol. 279, no. 23, pp. 24427–24434, 2004.

[59] K. A. Lillycrop, J. L. Slater-Jefferies, M. A. Hanson, K. M. Godfrey, A. A. Jackson, and G. C. Burdge, "Induction of altered epigenetic regulation of the hepatic glucocorticoid receptor in the offspring of rats fed a protein-restricted diet during pregnancy suggests that reduced DNA methyltransferase-1 expression is involved in impaired DNA methylation and changes in histone modifications," *British Journal of Nutrition*, vol. 97, no. 6, pp. 1064–1073, 2007.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 249849, 12 pages
doi:10.1155/2008/249849

Clinical study

Peroxisome Proliferator-Activated Receptor-γ Is a Potent Target for Prevention and Treatment in Human Prostate and Testicular Cancer

Masahide Matsuyama and Rikio Yoshimura

Department of Urology, Osaka City University Graduate School of Medicine, 1-4-3 Asahi-machi, Abeno-ku, Osaka 545-8585, Japan

Correspondence should be addressed to Rikio Yoshimura, jasmin@med.osaka-cu.ac.jp

Received 30 May 2007; Revised 13 August 2007; Accepted 6 October 2007

Recommended by P. Froment

Peroxisome proliferator-activated receptor-γ (PPAR)-γ is a ligand-activated transcriptional factor belonging to steroid receptor superfamily. PPAR-γ plays a role in both adipocyte differentiation and tumorigenesis. Up to date, PPAR-γ is expressed in various cancer tissues, and PPAR-γ ligand induces growth arrest of these cancer cells. In this study, we examined the expression of PPAR-γ in prostate cancer (PC) and testicular cancer (TC) by RT-PCR and immunohistochemistry, and we also examined the effect of PPAR-γ ligand in these cells by MTT assay, hoechest staining, and flow cytometry. PPAR-γ expression was significantly more extensive and intense in malignant tissues than in normal tissues. PPAR-γ ligand induced the reduction of malignant cell viability through apoptosis. These results demonstrated that the generated PPAR-γ in PC and TC cells might play an important role in the tumorigenesis. PPAR-γ may become a new target in the treatment of PC and TC.

1. INTRODUCTION

Prostate cancer (PC) comprises 32% of all cancers in American men and is on the increase worldwide. Because of increased screening, PC is frequently diagnosed at a clinically localized stage, making it amenable to the therapy. Nevertheless, it remains the second most common cause of cancer death in men. These patients generally respond to androgen deprivation therapy, but the vast majority eventually experience disease progression and become refractory to sustained hormonal manipulation. Typically, such patients progress with a rise in their serum prostate-specific antigen level. Unfortunately, standard therapeutic options at this stage of disease are limited, and while there has been some success with chemotherapy for hormone-refractory prostate cancer patients, the response is generally short lived [1].

Testicular cancer (TC) is very rare with over 90% of all TC being germ cell tumors (seminoma and nonseminoma), and the remaining percentage nongerminal tumors. The survival rate of TC has improved in recent years, reflecting the development and refinement of effective combination chemotherapy. However, it is still necessary to improve the treatment of TC.

Angiogenetic factors play an important role in prostate and testis as in other organs [2], and although various potential angiogenetic factors have been identified in PC and TC, it is still unclear by which process PC and TC cells become angiogenic. Thus, the challenge is to discover new treatment strategies that target androgen-independent PC and TC. The identification of molecular targets involved in the tumorigenesis and progression of PC and TC provide opportunities for the development of new agents with greater therapeutic potential and better specificity. Patients with advanced or recurrent disease are suitable candidates for studies that test the efficacy of these new agents.

Peroxisome proliferator-activated receptors (PPARs) are lipid-activated transcription factors that function as important regulars of lipid and glucose metabolism, adipocyte differentiation, and energy homeostasis. PPAR subtypes (α, β, and γ) have been found. Both PPAR-α and -γ mediate the

action of the hypolipidemic fibrates and antidiabetic thiazolidinediones. PPARs therefore play a role in metabolic conditions such as dyslipidemia and type 2 diabetes, leading to atherosclerosis development [3]. PPARs also have regulatory role in inflammation.

PPAR-γ provides a strong link between lipid metabolism and regulation of gene transcription [4]. PPAR-γ acts in adipose tissue and promotes lipogenesis under anabolic conditions. Recently, the receptor has also been implicated in inflammation and tumorigenesis. Significant evidence from many experimental systems suggests that PPAR-γ is important in carcinogenesis.

PPAR-γ is up regulated in malignant tissue, and PPAR-γ ligands induce terminal differentiation in human breast and colon cancer cells [5, 6], and inhibit the growth of human lung and gastric cancer cells [7, 8]. In addition, PPAR-γ ligands induce growth arrest through apoptosis in macropharge, fibrobrasts, and endothelial cells [3, 9, 10]. Our research elucidates the expression of PPARs in urological cancers and administration of PPAR-γ ligands as an anticancer therapy [11–15]. Several reports support the expression of PPAR-γ and the efficacy of PPAR-γ ligands in PC [16–18]. However, no further data on TC and PPAR-γ have been documented in other reports.

Our research focuses on the relationship between PPAR-γ and male reproductive system (prostate and testis) and on the anticancer effect of PPAR-γ ligands.

2. METHODS

2.1. Tumor specimens

Prostate specimens were obtained from 156 patients with PC; 15 with prostatic intraepithelial neoplasia (PIN); 20 with benign prostatic hyperplasia (BPH), who underwent biopsy due to serum prostate-specific antigen increase; and 12 patients with normal prostate (NP) tissues who underwent total cystectomy due to bladder cancer.

Testis specimens were obtained from 72 TC patients, and from 20 NT patients who underwent orchiectomy for PC. Tumor tissues, nontumor tissues, vascular endothelium, and interstitial tissues from the subjects were preserved in 10% formalin and embedded in paraffin, serially sectioned onto microscope slides at a thickness of 4 μm.

2.2. Antibodies

PPAR-α, -β, and -γ are affinity-purified goat polyclonal antibodies. We purchased these antibodies from Santa Cruz Biotechnology Inc (Santa Cruz, Calif, USA). They demonstrated about the source of these antibodies, PPAR-α and -γ are affinity-purified goat polyclonal antibodies raised against a peptide mapping at the amino terminus of PPAR-α and -γ of human origin (α differs from corresponding mouse sequence by amino acids; γ is identical to the corresponding mouse sequence). PPAR-β is an affinity-purified goat polyclonal antibody raised against a peptide mapping at the carboxy terminus of PPAR-β of human origin (differs from corresponding mouse sequence by two amino acids). About specificity of these antibodies, PPAR-α and -β react with those of mouse, rat and human origin by Western blotting and immunohistochemistry. PPAR-γ also reacts with PPAR-γ1 and PPAR-γ2 of mouse, rat, and human origin by Western blotting and immunohistochemistry. These specific antibodies do not cross-match either each other, nor do they cross-react with each other.

2.3. RT-PCR

Total RNA was isolated from PC tissues, BPH and NP tissues (fresh tissues) by guanidium thiocyanate-phenol-chloroform method. We performed an RT-PCR procedure to determine the PPAR-α, -β, and -γ mRNA expression as described previously [19]. In short, total RNA was used as a template for DNA synthesis using a superscript preamplification system (GIBCO-BRL) according to the manufacturer instructions. PCR was performed with each cDNA; PPAR-α, -β, and -γ; or G3PDH primer and Taq DNA polymerase (NIPPON GENE, Toyama, Japan). The synthetic oligonucleotides were obtained from Nippon Flour Mills (Kanazawa, Japan). We used G3PDH mRNA as a control.

The primers used were as follows:

(a) PPAR-α: sense; 5′-CCAGTATTTAGGACGCTGTCC-3′ and antisense 5′-AAGTTCTTCAAGTAGGCCA-GC-3′;
(b) PPAR-β: sense; 5′-AACTGCAGATGGGCTGTAAC-3′ and antisense 5′-GTCTCGATGTCGTGGATCAC-3′;
(c) PPAR-γ: sense; 5′-TCTCTCCGTAATGGAAGACC-3′ and antisense 5′-GCATTATGAGACATCCCCAC-3′;
(d) human G3PDH: sense; 5′-CCACCCATGGCAAATT-CCATGGCA-3′ and antisense; 5′-TCTAGAGGGC-AGGTCAGGTCCACC-3′.

The primer sets yield PCR products of 492, 484, 474, and 598 base pair for PPAR-α, -β, and -γ or G3PDH, respectively. Reactions were incubated in an automatic heat-block for 30 cycles of denaturation 40 seconds, 94°C ; annealing for 50 seconds, 50°C; extension for 50 seconds, 72°C [19]. PCR products were run on 2% agarose gel in TAE buffer (40 mM Tris acetate, 1 mM EDTA) and visualized by ethidium bromide staining.

2.4. Immunohistochemistry

Tissues sections (4 μm thick) were incubated with anti-PPAR-α, -β, and -γ antibody (2 μg/mL) or purified normal goat IgG (2 μg/mL) in a humid chamber for 24 hours, and further incubated with biotinylated rabbit antigoat IgG (Vector Laboratories, Inc. Burlingame, Calif, USA) for 30 minutes. After washing with PBS, the sections were incubated with the vectastatin avidin-biotin peroxidase complex kit (Vector, Burlingame, Calif, USA) [20] for 45 minutes. Color was developed by immersing the sections in a solutions of 0.05% wt/vol 3, 3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich, St. Louis, Mo, USA). The sections were counterstained with hematoxylin (Sigma-Aldrich, St. Louis, Mo, USA).

2.5. Statistical analysis

The extent and intensity of staining with PPAR-α, -β, and -γ antibodies were graded on a scale of 0 to 4 (+) by two blind observers on two separate occasions using coded slides, and an average score was calculated [21]. Staining was classified into 5 grades from 0 to 4 (+) according to the intensity of staining and the number of positive cells. The observers assessed all tissues on the slides to assign the score. A 4 (+) grade implies that all staining was maximally intense throughout the specimen, whereas 0 implies that staining was absent throughout the specimen. The microanatomical sites of staining were also recorded. To quantify the expressions of PPAR-α, -β, and -γ, the same two pathologists made assessments throughout the study, staining control specimens simultaneously. This method, therefore, increases the credibility of data. In addition, all specimens were reassessed, which also contributed to the exclusion of any subjective variability.

2.6. Cell cultures

The human PC cell lines (LNCaP, PC3, DU-145) and TC cell line (NEC-8) were obtained from Health Science Research Resources Bank (Osaka, Japan). Cells were grown in culture flask (Nunc, Roskilde, Denmark) in RPMI 1640 supplemented with 10% FBS, 100 U/mL of penicillin and 100 μg/mL of streptomycin in a humidified 5% CO_2 atmosphere at 37°C. The media were changed every 3 days and the cells were separated via trypsinization, using trypsin/EDTA when they reached subconfluence.

2.7. Cell proliferative studies

Troglitazone (thiazolidinedione compounds) was obtained from Sankyo Pharmaceuticals (Tokyo, Japan). 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15-d-PGJ_2) was purchased from Cayman Chemical Company (St. Louis, Mo, USA). GW9662 was purchased from BIOMOL Research Laboratories Inc. (Pa, USA). Approximately 1.0×10^4 cells (all PC and TC cell lines) placed onto 8×8 mm diameter multichamber slides (Nunc, Copenhagen, Denmark) were treated with troglitazone and 15-d-PGJ_2 (5–40 μM) dissolved in ethanol. The final concentration of ethanol was <0.05%. Cell viability was measured after 48 hours by a microplate reader using a modified 3-[4,5-dimethylthiazol-2-thiazolyl]-2,5-diphenyltetrazolium bromide (MTT) assay (WST-1 assay; Dojindo, Kumamoto, Japan) and presented as the percentage of control-culture conditions ($N = 6$).

2.8. Flow cytometry (annexin V and propidium iodide staining)

The effects of PPAR-γ ligands on PC (PC3) and TC (NEC-8) cell lines were determined by dual staining with Annexin V-FITC and propidium iodide using Annexin V-FITC Apoptosis Detection Kit I (Biosciences Pharmingen, Calif, USA). Annexin V-FITC and propidium iodide (PI) were added to the cellular suspension as in the manufacturer instruction,

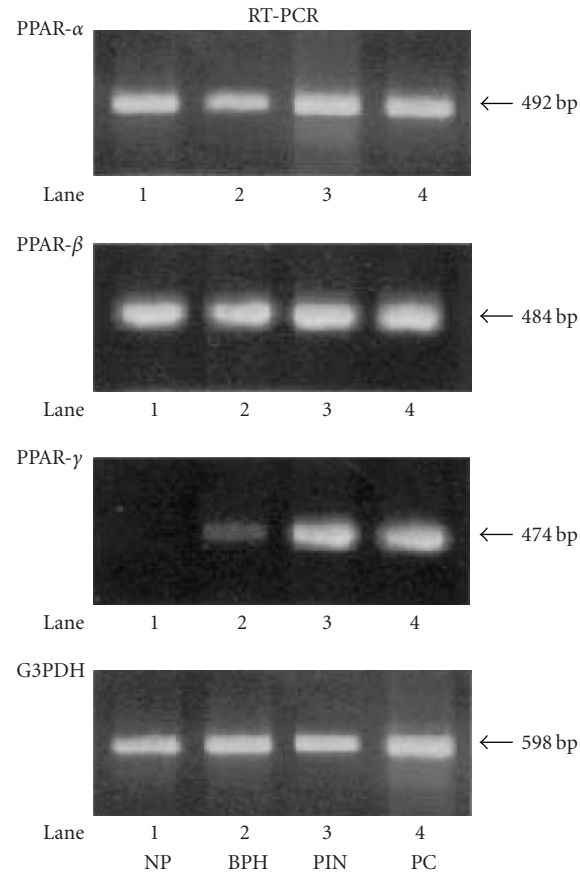


Figure 1: RT-PCR analysis of PPAR-α, -β, and -γ in prostate tissue samples from the patients with PC, PIN, BPH and NP. A slight, but clear, band of PPAR-α and -β m-RNA was detected in all samples. However, the specific band of PPAR-γ mRNA in the samples from prostate cancer (PC) and prostatic intraepithelial neoplasia (PIN) was detected, while samples from benign prostatic hyperplasia (BPH) displayed a very weak band, and in a sample from normal prostate (NP) no clear band was detected.

and sample fluorescence of 1.0×10^4 cells was analyzed flow cytometry. Flow cytometry was with FACScan (Becton Dickinson, Heidelberg, Germany). Cell which were Annexin V-FITC positive and PI negative were identified as early apoptosis. Cell which were Annexin V-FITC positive and PI positive were identified as late apoptosis or necrosis.

2.9. Flow cytometry (identification of DNA fragmentation)

The assay was performed by TdT-mediated dUTP Nick End Labelling (TUNEL) method using APO-DIRECT kit (Becton Dickinson). Following the experiments, PC (PC3) and TC (NEC-8) cell lines in suspension (1×10^6/mL) were fixed with 1% PBS, washed in PBS, and suspended in 70% (v/v) ice-cold ethanol. The cells were stored in ethanol at −20°C until use. The positive and negative controls and the sample were stained with FITC-dUTP by incubation in terminal deoxynucleotidyl transferase buffer as in the manufacturer instruction, and sample fluorescence of 1×10^4 cells

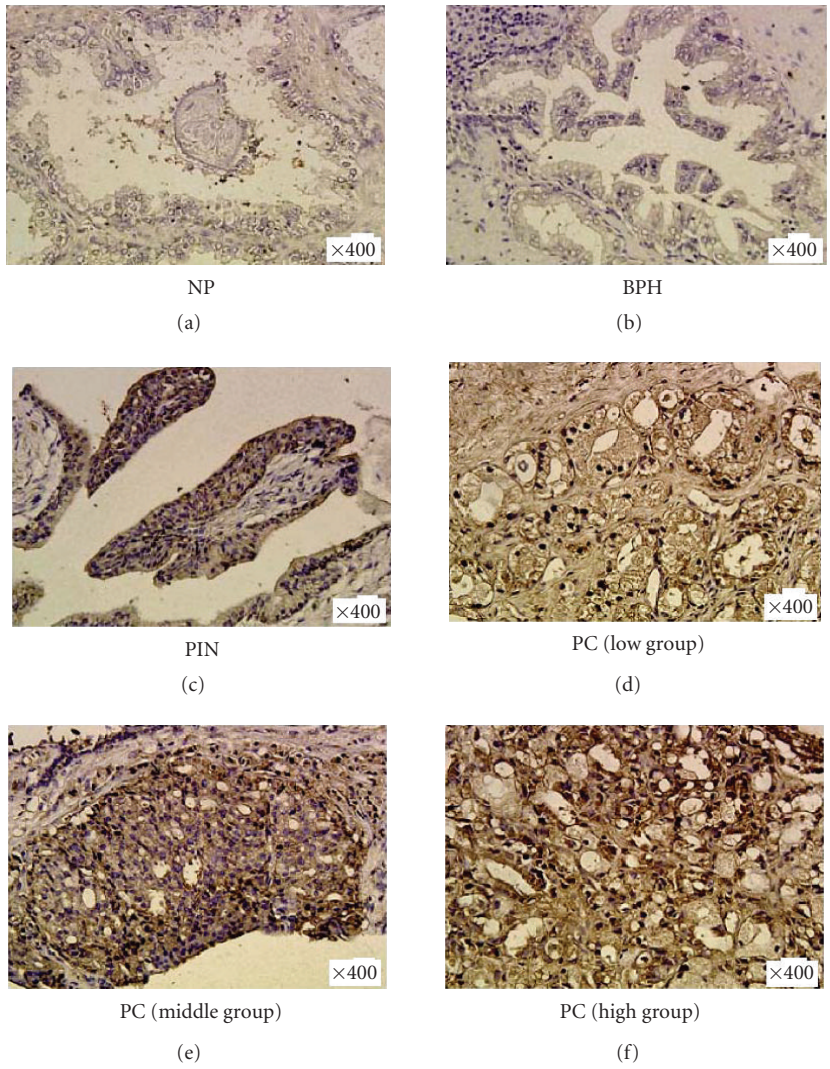


Figure 2: Representative immunostaining for PPAR-γ in prostate tissues samples. A significant strong PPAR-γ expression in all prostate cancer (PC) group tissues and prostatic intraepithelial neoplasia (PIN) tissue was detected, whereas PPAR-γ expression is very weak in benign prostatic hyperplasia (BPH) tissues and normal prostate (NP) tissue.

was analyzed by flow cytometry (Becton Dickinson). Results are given as % of TUNEL-positive cells.

2.10. Detection of apoptosis by Hoechst staining

DNA chromatin morphology was assessed using Hoechst staining. PC (PC3) and TC (NEC-8) cell (5×10^5 cells) were incubated with 20 μM PPAR-γ ligands for 24 hours. Cells were washed by RPMI-1640 and labeled with 8 mg/mL of hoechest 33342 (Sigma-Aldrich Japan K.K. Tokyo, Japan) for 10 minutes; PI (Sigma-Aldrich Japan K.K. Tokyo, Japan) was added (10 mg/mL final concentration), and the cells were examined by fluorescence microscopy.

2.11. Statistical analysis

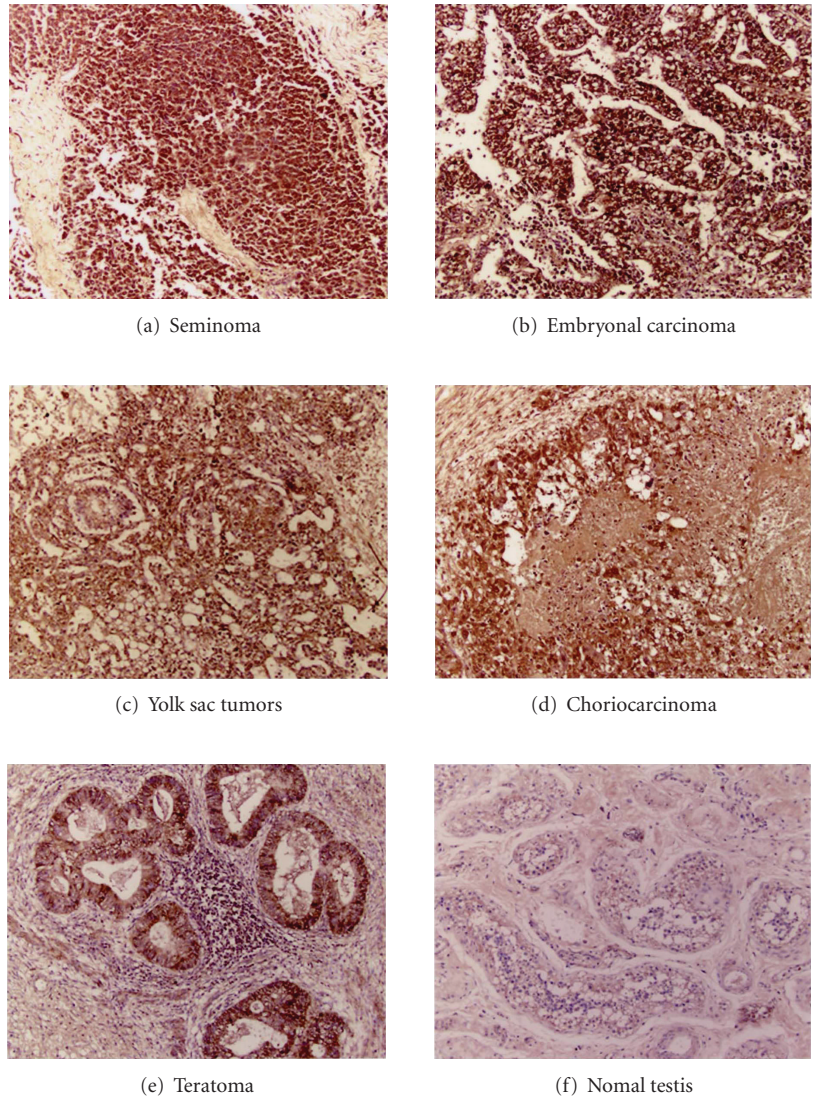
All results are presented as the mean ± SD. Analysis of data was performed using the analysis of variance (ANOVA) [22].

3. RESULTS

3.1. Tumor specimens

3.1.1. PC tissue sample

The 156 patients with PC were male aged 59–78 years (mean age 67 ± 5.3 years). We used Gleason score to evaluate PC. Gleason score is given to PC based upon its microscopic appearance. Gleason score is important because higher Gleason scores are associated with worse prognosis. This is because higher Gleason scores are given to cancer which is more aggressive. Gleason score ranges from 2 to 10. Gleason score of 2 is associated with the best prognosis and a score of 10 with the worst. The final score is a combination of two different scores which each range from 1 to 5. Gleason score is as follows: low group: Gleason score, 2, 3, 4, 5, middle group: Gleason score, 6, 7, 8, high group: Gleason score, 9, 10. In clinical PC, Gleason score is almost over 5.



(a) Seminoma (b) Embryonal carcinoma

(c) Yolk sac tumors (d) Choriocarcinoma

(e) Teratoma (f) Nomal testis

Figure 3: Representative immunostaining for PPAR-γ in testicular tissues samples. A significant strong PPAR-γ expression in all testicular cancer (TC) tissues was detected, whereas PPAR-γ expression is very weak in normal testis (NT) tissues.

The 50 patients were in the low group, 54 were in the middle group, and 52 were in the high group. The 15 patients with PIN averaged 64 ± 5.9 (52–73) years. The 20 patients with BPH averaged 68 ± 4.7 years (59–75), and all had nodular hyperplasia. The 12 patients with NP averaged 52 ± 7.6 (44–62) years.

3.1.2. TC tissue sample

The 72 TC patients were a mean age of 31.0 ± 12.3 years. Tumors of single histologic types were found in 58 patients and more than two histological types in 14 patients. Seminoma occurred in 31 patients, embryonal carcinoma in 8 patients, yolk sac tumor in 7 patients, choriocarcinoma in 7 patients, and teratoma in 5 patients. Tumors having more than two histologic types included embryonal carcinoma and teratoma in 4 patients, choriocarcinoma and other types in 3 patients, and other combinations in 7 patients. The average age of 20 patients NT tissues was 61.4 ± 8.6 years.

3.2. RT-PCR

To check PPAR-α, -β, and -γ mRNA variation, RT-PCR was performed with total RNA extracted from all specimens. Using specific primers for PPAR-α, -β, and -γ and G3PDH, the amplification predicted, respectively, fragments of 492, 484, 474, and 598 base pair (bp) in length.

3.2.1. PC tissue sample

The PPAR-α and -β mRNA were detected in PC, PIN, BPH, and NP samples. However, we detected a specific band of PPAR-γ mRNA in the samples from PIN and PC, and we also detected a very weak specific band of PPAR-γ mRNA in

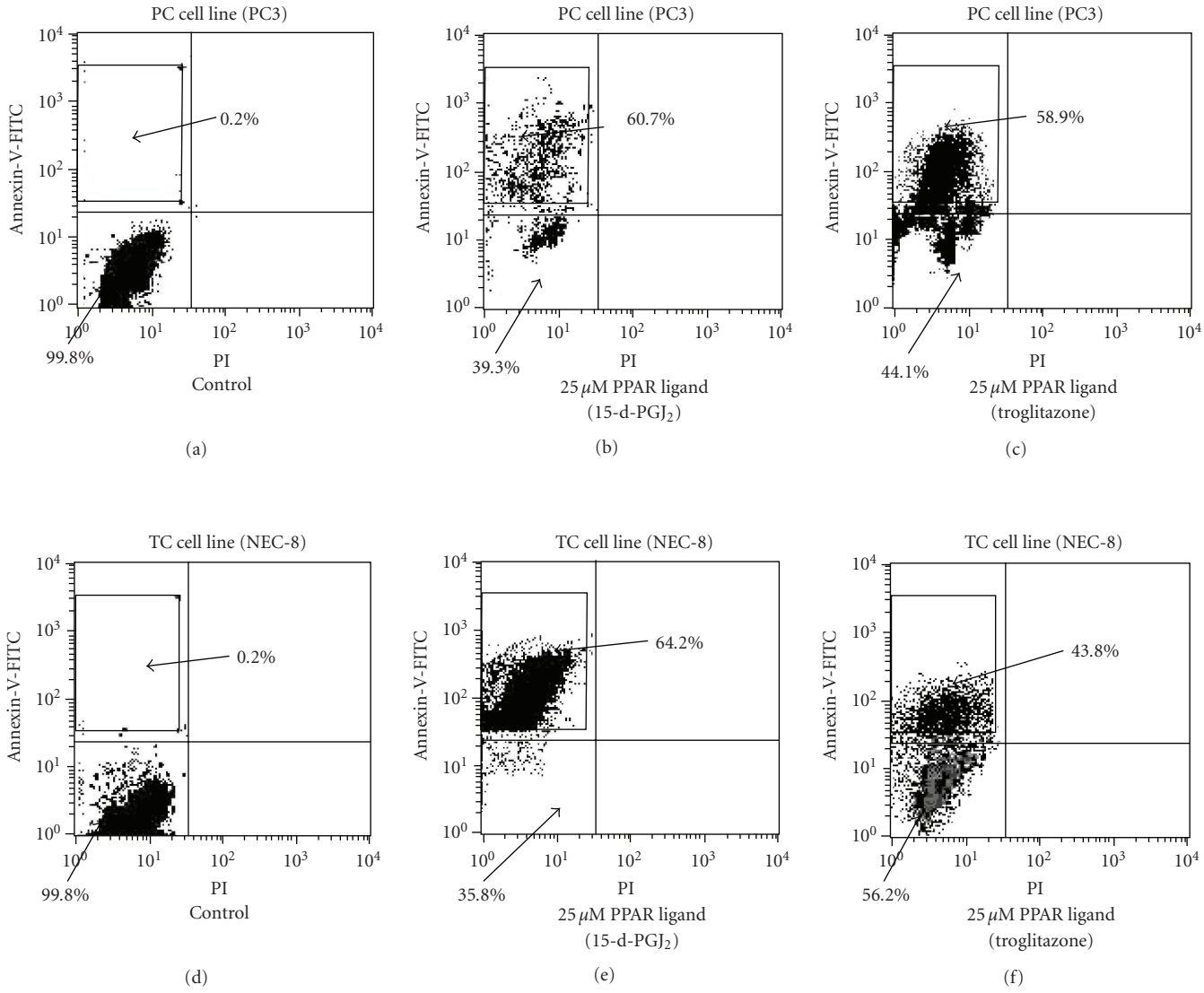


Figure 4: Effects of PPAR-γ ligands on apoptosis by flow cytometry in PC and TC cell lines. PC cells (PC3) and TC cell (NEC-8) lines with treatment of 25 μM 15-d-PGJ$_2$ could induce early apoptosis not late apoptosis or necrosis. The higher left quadrants represent early apoptosis (Annexin V-FITC-positive cells and PI-negative cells). The higher right quadrants represent late apoptosis or necrosis (Annexin V-FITC-positive cells and PI-positive cells). Diagrams of FITC-Annexin V/PI flow cytometry are presented.

the sample from BPH, whereas sample from NP displayed no band of PPAR-γ mRNA (see Figure 1).

3.2.2. TC tissue sample

The PPAR-α and -β mRNA were detected in all TC and NT samples. However, we detected a specific band of PPAR-γ mRNA in all TC groups, whereas sample from NT displayed no band of PPAR-γ mRNA.

3.3. Immnohistostaining of PPAR-α, -β, and -γ

To assess the tissue distribution of PPAR-α, -β, and -γ polypeptides, we stained paraffin-embedded samples with the affinity-purified PPAR-α, -β, and -γ antibodies that recognize specifically PPAR-α, -β, and -γ. The specificity of this antibody was proved by the previous experiments [23].

3.3.1. PC tissue sample

PPAR-α, and -β were expressed in PC, PIN, BPH, and NP tissues. Although very weak expression of PPAR-γ was found in BPH and NP tissues, PPAR-γ was strongly expressed in all PC groups and PIN (see Figure 2).

3.3.2. TC tissue sample

PPAR-α, and -β were expressed in all TC and NT tissues. Although no expression of PPAR-γ was found in NT tissues, PPAR-γ was strongly expressed in all TC groups (see Figure 3).

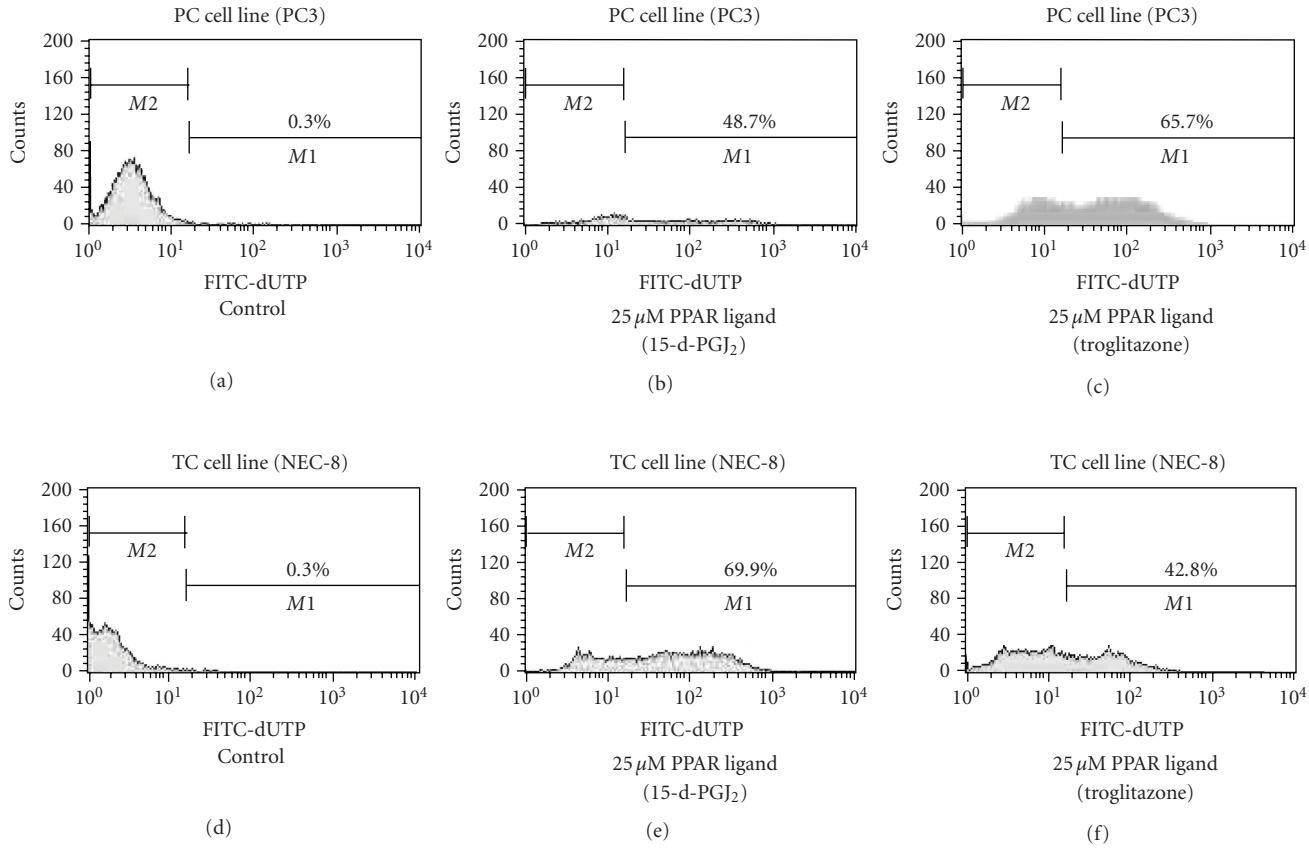


Figure 5: PPAR-γ ligands induce DNA fragmentation in PC and TC cell lines. 15-d-PGJ_2 (2 μM) could induce DNA fragmentation in PC cell (PC3) and TC cell line (NEC-8). Typical flow cytometry analysis histograms are presented.

3.4. Statistical analysis of PPAR-α, -β, and -γ immunostaining

To extent and intensity of staining with PPAR-α, -β, and -γ, antibody was graded 0 to 4 (+) by 2 blind observers.

3.4.1. PC tissue sample

PPAR-α, -β immunostaining were significantly intense in all cases. There were no differences among PC, PIN, BPH, and NP. There was no significant difference of the intensity of PPAR-α, -β staining between PC, PIN, BPH, and NP. However, PPAR-γ immunostaining was significantly more extensive and intense in tumor cells (mean: low group; 2.6 ± 0.7, middle group; 2.7 ± 0.9, high group; 3.3 ± 1.0, $P < .01$) and in PIN (mean: 2.5 ± 0.8, $P < .01$) than in tissue of BPH (mean: 0.8 ± 0.6). PPAR-γ staining was also high in blood vessels and stromal tissues of prostate cancer and PIN, with no significant difference between them (1.8–2.0). However, the expression of the PPAR-γ in the blood vessels and stromal tissues from BPH and NP was at the basic level (0.5–0.7) (see Table 1).

3.4.2. TC tissue sample

PPAR-α, -β immunostaining were significantly intense in all TC groups and NT. However, PPAR-γ immunostaining was significantly more extensive and intense in tumor cells and blood vessels of the TC groups than in NT. There was no significant differences occurred between all TC group in tumor cells and blood vessels (see Table 2).

3.5. PPAR-γ ligands induced growth inhibition in PC and TC cell lines by MTT assay

To investigate the effects of PPAR-γ ligands on all PC (LNCaP, PC3, DU-145) and TC cell (NEC-8) lines proliferation, we analyzed cell viability in vitro by modified MTT assay.

3.5.1. PC cell line

PPAR-γ ligands induced the reduction of cell viability with the half-maximal concentration of growth inhibition of all PC cell lines (LNCaP, PC3, DU-145) in the range of 5–40 μM (see Table 3). PPAR-γ ligands stopped the growth of all PC cell lines.

3.5.2. TC cell line

Similar to PC cell lines, PPAR-γ ligands induced the reduction of cell viability with the half-maximal concentration of growth inhibition of TC cell line (NEC-8) in the range of

Table 1: Statistical analysis of PPAR-α, -β, and -γ immunostaining.

		Tumor	Blood vessel	Stromal tissue
PPAR-α	PC ($N = 156$)			
	Low group $N = 50$)	2.2 ± 0.6	2.0 ± 0.9	2.0 ± 0.9
	Middle group $N = 54$)	2.4 ± 0.7	2.3 ± 0.7	1.9 ± 0.6
	High group $N = 52$)	2.3 ± 0.7	1.9 ± 0.6	2.0 ± 0.8
	PIN ($N = 15$)	1.9 ± 0.6	1.8 ± 0.6	1.7 ± 0.7
	BPH ($N = 20$)	2.1 ± 0.6	1.7 ± 0.5	1.7 ± 0.5
	NP ($N = 12$)	Not present	2.1 ± 0.7	2.2 ± 1.1
PPAR-β	PC ($N = 156$)			
	Low group $N = 50$)	2.0 ± 1.0	1.7 ± 0.7	1.9 ± 0.6
	Middle group $N = 54$)	2.3 ± 1.2	1.6 ± 0.7	1.8 ± 0.7
	High group $N = 52$)	2.1 ± 1.0	1.7 ± 0.6	1.8 ± 0.9
	PIN ($N = 15$)	2.2 ± 0.8	1.8 ± 0.9	1.8 ± 0.7
	BPH ($N = 20$)	2.0 ± 0.7	1.9 ± 0.8	1.6 ± 0.7
	NP ($N = 12$)	Not present	1.9 ± 0.7	1.9 ± 0.8
PPAR-γ	PC ($N = 156$)			
	Low group $N = 50$)	2.6 ± 0.7*	1.8 ± 0.9*	1.8 ± 0.7*
	Middle group $N = 54$)	2.7 ± 0.9*	1.8 ± 0.8*	1.9 ± 0.9*
	High group $N = 52$)	3.3 ± 1.0*	2.0 ± 0.8*	1.7 ± 0.9*
	PIN ($N = 15$)	2.5 ± 0.8*	1.9 ± 0.8*	1.7 ± 0.9*
	BPH ($N = 20$)	0.8 ± 0.6	0.6 ± 0.5	0.7 ± 0.5
	NP ($N = 12$)	Not present	0.5 ± 0.4	0.5 ± 0.3

* Graded 0–4 on the coded sections by two blind observers. 0, no staining; 4+, maximum intensity. Statistical analysis was performed using the ANOVA (p-value). PPAR-α and -β immunostaining were significantly intense in all cases. There were no differnces among PC, PIN, BPH and NP. PPAR-γ immunostainings of tumor were significantly more extensive and intense in PC and in PIN than in tissue of BPH or NP. PPAR-γ staining was high in blood vessels and stromal tissues of PC and PIN, with no significant difference between them. However, the expressions of the PPAR-γ in the blood vessels and stromal tissues from BPH and NP were at the basic level. (*$P < .01$).

5–40 μM (see Table 3). PPAR-γ ligands stopped the growth of TC cell line (NEC-8).

3.6. PPAR-γ ligands induced apoptosis by flow cytometry

To evaluate whether or not cell death induced by PPAR-γ ligands was through apoptosis, we evaluated using flow cytometry. The higher left quadrants represent early apoptosis (Annexin V-FITC-positive cells and PI-negative cells). The higher right quadrants represent late apoptosis or necrosis (Annexin V-FITC-positive cells and PI-positive cells).

3.6.1. PC cell line

PC cell line (PC3) with treatment of 25 μM PPAR-γ ligand (15-d-PGJ_2) could induce early apoptosis, not late apoptosis or necrosis (see Figure 4) and DNA fragmentation (see Figure 5). Diagrams of FITC-Annexin V/PI flow cytometry and typical flow cytometry analysis histogram are presented.

3.6.2. TC cell line

TC cell line (NEC-8) with treatment of 25 μM PPAR-γ ligands (15-d-PGJ_2) could induce early apoptosis not late apoptosis or necrosis (see Figure 4) and DNA fragmentation (see Figure 5). Diagrams of FITC-Annexin V/PI flow cytometry and typical flow cytometry analysis histogram are presented.

3.7. Effect of PPAR-γ ligands in induction of apoptosis on PC and TC cell lines

To evaluate whether or not cell death induced by PPAR-γ ligands was through apoptosis, we evaluated the chromatin morphology of PC (PC3) cell and TC cell (NEC-8) lines using hoechst staining.

3.7.1. PC cell line

PC cell line (PC3) treated with PPAR-γ ligands showed significant chromatin condensation, cellular shrinkage, small membrane-bound bodies (apoptotic bodies), and cytoplasmic condensation. These cellular changes were typically redundant characteristics of apoptosis. PC cell lines (PC3) without PPAR-γ ligands maintained normal chromatin patterns and cell size (see Figure 6). Typical photographs are presented in Figure 6.

3.7.2. TC cell line

Similar to PC cell line, TC cell line (NEC-8) treated with PPAR-γ ligands showed significant chromatin condensation,

Table 2: Statistical analysis of PPAR-α, -β, and -γ immunostaining.

		Av.± SD	
	Tumor type	Epithelium	Blood vessel
PPAR-α	Seminoma ($N = 34$)	2.2 ± 0.8	1.7 ± 0.9
	Embryonal carcinoma ($N = 15$)	2.4 ± 1.1	2.1 ± 1.0
	Yolk sac tumor ($N = 11$)	1.8 ± 1.0	1.6 ± 0.7
	Choriocarcinoma ($N = 10$)	2.5 ± 1.2	2.0 ± 0.9
	Teratoma ($N = 12$)	1.8 ± 0.9	1.6 ± 0.9
	Normal testis ($N = 20$)	2.5 ± 1.1	2.1 ± 0.9
PPAR-β	Seminoma ($N = 34$)	2.4 ± 0.9	2.2 ± 1.1
	Embryonal carcinoma ($N = 15$)	2.6 ± 1.4	2.3 ± 1.2
	Yolk sac tumor ($N = 11$)	2.5 ± 1.4	2.1 ± 0.6
	Choriocarcinoma ($N = 10$)	2.2 ± 1.0	1.9 ± 0.9
	Teratoma ($N = 12$)	2.4 ± 0.9	2.2 ± 1.3
	Normal testis ($N = 20$)	2.5 ± 1.1	2.3 ± 1.0
PPAR-γ	Seminoma ($N = 34$)	2.2 ± 0.8*	1.9 ± 0.9*
	Embryonal carcinoma ($N = 15$)	2.8 ± 1.1*	2.5 ± 1.0*
	Yolk sac tumor ($N = 11$)	2.2 ± 0.9*	2.1 ± 1.1*
	Choriocarcinoma ($N = 10$)	2.9 ± 1.0*	2.4 ± 1.0*
	Teratoma ($N = 12$)	2.0 ± 1.3*	1.9 ± 1.1*
	Normal testis ($N = 20$)	0.7 ± 0.6	0.6 ± 0.4

* Graded 0 to 4 on the coded sections by two blind observers. 0, no staining; 4+, maximum intensity. Statistical analysis was performed using the analysis of variance (P value; ANOVA). PPAR-α, and -β immunostaining were significanty apparent in all TC and NT tissues. PPAR-γ immunostaining of tumor was significantly more extensive and intense in all TC groups than in NT tissue. PPAR-γ staining was high in blood vesseld of TC, with no significant difference between them. However, the expression of PPAR-γ in blood vessels from NT was at the basic level. $P < .01$.

Table 3: Effects of troglitazone, 15-d-PGJ2 and GW9662 in viabity of human PC and TC cell lines.

		5 μM	10 μM	20 μM	40 μM
Troglitazone					
PC cell lines	LNCaP	72.4%	25.7%	12.6%	8.4%
	PC3	48.6%	15.5%	14.7%	6.5%
	DU-145	60.1%	35.1%	7.6%	7.7%
TC cell line	NEC-8	38.7%	35.3%	36.6%	38.1%
15-d-PGJ2					
PC cell lines	LNCaP	78.9%	63.7%	22.4%	5.6%
	PC3	69.7%	59.0%	34.1%	6.8%
	DU-145	73.8%	59.3%	5.8%	5.8%
TC cell line	NEC-8	75.1%	66.7%	52.3%	46.8%
GW9662					
PC cell lines	LNCaP	106.8%	112.4%	103.7%	106.2%
	PC3	116.8%	118.6%	119.4%	120.2%
	DU-145	122.6%	119.4%	117.8%	115.6%
TC cell line	NEC-8	108.4%	115.5%	110.6%	112.3%

The dose-response analysis of viability in human cancer cells treated with troglitazone, 15-d-PGJ2 and GW9662 (5–40 μM, 48 hr) was measured by the MTT assay and expressed as % of control culture conditions ($N = 6$).

cellular shrinkage, small membrane-bound bodies (apoptotic bodies), and cytoplasmic condensation. These cellular changes were typically redundant characteristics of apoptosis. TC cell line without PPAR-γ ligands maintained normal chromatin patterns and cell size.

4. DISCUSSION

PPAR-α is highly expressed in the liver, heart, kidney, muscle, brown adipose tissue, and gut, which exhibit high carbolic rates of fatty acid. PPAR-β may be expressed ubiquitously

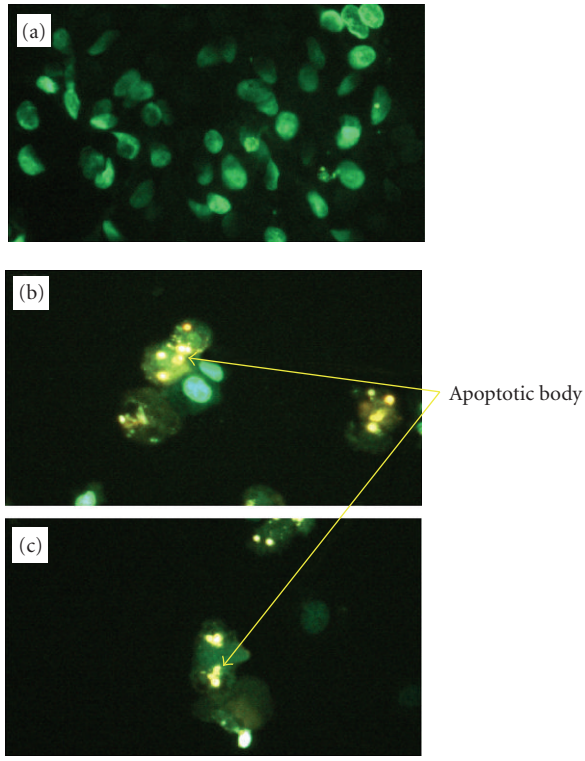


Figure 6: Effects of PPAR-γ ligands in induction of apoptosis on human PC cell line. PC cell line (PC3) treated with PPAR-γ ligands ((b); 20 μM Troglitazone, (c); 20 μM 15-d-PGJ_2) showed significant chromatin condensation, cellular shrinkage, small membrane-bound bodies (apoptotic bodies), and cytoplasmic condensation. These cellular changes were typically redundant characteristics of apoptosis. PC cells without PPAR-γ ligands maintained normal chromatin patterns and cell size (a). Typical photographs are presented.

and its function is relatively unknown. Recent studies suggest that PPAR-β may be a target for nonsteroidal antiinflammatory drugs (NSAIDs)-induced tumor suppression in colorectal tumors. PPAR-γ is expressed at high level in adipose tissue and is a critical regulator of adipocyte differentiation. In addition, PPAR-α, and -γ have been considered important immunomodulatory factors. PPAR-α knockout mice exhibit exacerbated inflammatory responses, and leukotriene B_4, a chemotractic mediator, appears to regulate the clearance of itself as an agonist of PPAR-α. However, PPAR-γ is also expressed in the immune system, in the spleen monocytes, bone-marrow precursors, and helper T-cell clones. PPAR-γ is also expressed in chondrocytes as well as in synovial and bone tissues. Recent data have shown that PPAR-γ ligands lead to inhibition of phorbol ester-induced nitric oxide and macropharge-derived cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), chemokines, and adhesion molecules, in part by antagonizing the activities of transcriptional factors [7].

Recently, it has been evidenced that thiazolidinedione, a new class of antidiabetic as a specific ligand for PPAR-γ, and retinoic receptor agonists can regulate differentiation of cancer cells [24], and that nuclear-acting prostanoids, including 15-d-PGJ_2, are potent activators of the PPAR-γ receptor isoform [25, 26]. In fact, 15-d-PDJ_2 induces apoptosis in macropharge, endothelial cell, choriocarcinoma cell [3, 10, 27], as well as thiazolidinediones-induced fibroblast apoptosis [9]. PPAR-γ ligands also inhibit vascular endothelial cell growth factor-induced angiogenesis in vivo [28]. Angiogenesis is important for tumorigenesis. Antiangiogenetic therapy is highly promising since it does not induce aquired anticancer drug resistance [29, 30]. Drevs et al. demonstrated the effect of PTK787/ZK 222584, a specific inhibitor of vascular endothelial growth factor receptor tyrosine kinases, on primary tumor, metastasis, vessel density, and blood flow in an animal model of renal cell carcinoma [31]. PPAR-γ agonists induce apoptosis in endothelial cells and inhibit vascular endothelial growth factor-induced angiogenesis in rats. Therefore, PPAR-γ ligands may have anticancer effects through inhibition of cell proliferation and angiogenesis.

In this time, concerning about PC, we demonstrated a stronger expression of PPAR-γ in PC and PIN tissues than in BPH or NP tissues by immunohistochemical staining and RT-PCR. We classified 3 categories (epithelial cells, blood vessels, and stromal tissues) in PC, PIN, BPH, and NP tissues, and examined the intensity of PPAR-α, -β, and -γ expressions in all tissue categories. There were no significant differences in the intensity of PPAR-α and -β in PC, PIN, BPH, and NP tissues. However, in all categories, PPAR-γ expression was significantly more extensive and intense in PC and PIN tissues than in BPH and NP tissues, and PPAR-γ expression was higher in G3 cancer than in G1 cancer. Paltoo et al. demonstrated that there were no significant differences between PPAR-γ expression in grades and stages [16]. Using competitive PCR, these differences may be demonstrated in the near future.

Next, we demonstrated that PPAR-γ ligands induced reduction of the viability in PC cells in the range of 5–40 μM by using MTT assay. Furthermore, we also demonstrated that PC cells treated with PPAR-γ ligands could induce early apoptosis and DNA fragmentation in PC cells. Subbarayan et al. have also demonstrated similar results [17]. Several reports support the efficacy of PPAR-γ ligands in PC [16, 18]. We expect that additional research will be progressed.

Concerning about TC, we demonstrate stronger expression of PPAR-γ in all tissue types of TC than in normal testicular tissues by immunohistochemical staining and RT-PCR. There were no significant differences among 5 histopathologic groups. We classified 2 categories (epithelial cells and blood vessels) in TC and NT tissues, and examined the intensity of PPAR-α, -β, and-γ expression. There were no significant differences in the intensity of PPAR-α, -β expression between all categories of TC and NT tissues. However, PPAR-γ expression was significantly more extensive and intense in all categories of TC than in NT tissues. Next, we demonstrated that PPAR-γ ligands induced the reduction of viability in TC cells in the range of 5–40 μM by MTT assay. Furthermore, we also demonstrated that TC cells treated with PPAR-γ ligands could induce early apoptosis and DNA fragmentation in TC cells. However, no further data on TC and PPAR-γ have been documented in other reports. We expect additional research will be progressed.

In summary, PPAR-γ expression was significantly more extensive and intense in malignant tissues than in normal tissue, and PPAR-γ expression was higher in G3 cancer than in G1 cancer. Furthermore, PPAR-γ ligands induced the reduction of malignant cell viability through apoptosis in vitro. These results indicate that PPAR-γ participates in initiation and promotion of tumorigenesis.

These results raise the possibility that PPAR-γ may play role in the pathogenesis and progression of PC and TC. While it is difficult at this time to use PPAR-γ ligands at a clinical dose (relatively nontoxic therapeutic approach) as suppressive cancer therapy, we strongly suggest that further research may confirm PPAR-γ ligands as a novel approach to the treatment of PC and TC.

REFERENCES

[1] W. K. Oh and P. W. Kantoff, "Management of hormone refractory prostate cancer: current standards and future prospects," *The Journal of Urology*, vol. 160, no. 4, pp. 1220–1229, 1998.

[2] S. Bosari, A. K. C. Lee, R. A. DeLellis, B. D. Wiley, G. J. Heatley, and M. L. Silverman, "Microvessel quantitation and prognosis in invasive breast carcinoma," *Human Pathology*, vol. 23, no. 7, pp. 755–761, 1992.

[3] G. Chinetti, S. Griglio, M. Antonucci, et al., "Activation of proliferator-activated receptors α and γ induces apoptosis of human monocyte-derived macrophages," *Journal of Biological Chemistry*, vol. 273, no. 40, pp. 25573–25580, 1998.

[4] B. M. Spiegelman, "PPAR-γ: adipogenic regulator and thiazolidinedione receptor," *Diabetes*, vol. 47, no. 4, pp. 507–514, 1998.

[5] E. Mueller, P. Sarraf, P. Tontonoz, et al., "Terminal differentiation of human breast cancer through PPARγ," *Molecular Cell*, vol. 1, no. 3, pp. 465–470, 1998.

[6] P. Sarraf, E. Mueller, D. Jones, et al., "Differentiation and reversal of malignant changes in colon cancer through PPARγ," *Nature Medicine*, vol. 4, no. 9, pp. 1046–1052, 1998.

[7] Y. Tsubouchi, H. Sano, Y. Kawahito, et al., "Inhibition of human lung cancer cell growth by the peroxisome proliferator-activated receptor-γ agonists through induction of apoptosis," *Biochemical and Biophysical Research Communications*, vol. 270, no. 2, pp. 400–405, 2000.

[8] N. Takahashi, T. Okumura, W. Motomura, Y. Fujimoto, I. Kawabata, and Y. Kohgo, "Activation of PPAR inhibits cell growth and induces apoptosis in human gastric cancer cells," *FEBS Letters*, vol. 455, no. 1-2, pp. 135–139, 1999.

[9] S. Altiok, M. Xu, and B. M. Spiegelman, "PPARγ induces cell cycle withdrawal: inhibition of E2f/DP DNA-binding activity via down-regulation of PP2A," *Genes & Development*, vol. 11, no. 15, pp. 1987–1998, 1997.

[10] D. Bishop-Bailey and T. Hla, "Endothelial cell apoptosis induced by the peroxisome proliferator- activated receptor (PPAR) ligand 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Journal of Biological Chemistry*, vol. 274, no. 24, pp. 17042–17048, 1999.

[11] K.-I. Inoue, Y. Kawahito, Y. Tsubouchi, et al., "Expression of peroxisome proliferator-activated receptor γ in renal cell carcinoma and growth inhibition by its agonists," *Biochemical and Biophysical Research Communications*, vol. 287, no. 3, pp. 727–732, 2001.

[12] R. Yoshimura, M. Matsuyama, Y. Segawa, et al., "Expression of peroxisome proliferator-activated receptors (PPARs) in human urinary bladder carcinoma and growth inhibition by its agonists," *International Journal of Cancer*, vol. 104, no. 5, pp. 597–602, 2003.

[13] Y. Segawa, R. Yoshimura, T. Hase, et al., "Expression of peroxisome proliferator-activated receptor (PPAR) in human prostate cancer," *The Prostate*, vol. 51, no. 2, pp. 108–116, 2002.

[14] T. Hase, R. Yoshimura, M. Mitsuhashi, et al., "Expression of peroxisome proliferator-activated receptors in human testicular cancer and growth inhibition by its agonists," *Urology*, vol. 60, no. 3, pp. 542–547, 2002.

[15] R. Yoshimura, M. Matsuyama, T. Hase, et al., "The effect of peroxisome proliferator-activated receptor-gamma ligand on urological cancer cells," *International Journal Of Molecular Medicine*, vol. 12, no. 6, pp. 861–865, 2003.

[16] D. Paltoo, K. Woodson, P. Taylor, D. Albanes, J. Virtamo, and J. Tangrea, "*Pro12Ala* polymorphism in the peroxisome proliferator-activated receptor-gamma (PPAR-γ) gene and risk of prostate cancer among men in a large cancer prevention study," *Cancer Letters*, vol. 191, no. 1, pp. 67–74, 2003.

[17] V. Subbarayan, A. L. Sabichi, J. Kim, et al., "Differential peroxisome proliferator-activated receptor-γ isoform expression and agonist effects in normal and malignant prostate cells," *Cancer Epidemiology Biomarkers & Prevention*, vol. 13, no. 11, pp. 1710–1716, 2004.

[18] R. Butler, S. H. Mitchell, D. J. Tindall, and C. Y. F. Young, "Nonapoptotic cell death associated with S-phase arrest of prostate cancer cells via the peroxisome proliferator-activated receptor γ ligand, 15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2," *Cell Growth & Differentiation*, vol. 11, no. 1, pp. 49–61, 2000.

[19] D. Auboeuf, J. Rieusset, L. Fajas, et al., "Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients," *Diabetes*, vol. 46, no. 8, pp. 1319–1327, 1997.

[20] H. Sano, K. Engleka, P. Mathern, et al., "Coexpression of phosphotyrosine-containing proteins, platelet-derived growth factor-B, and fibroblast growth factor-1 in situ in synovial tissues of patients with rheumatoid arthritis and Lewis rats with adjuvant or streptococcal cell wall arthritis," *The Journal of Clinical Investigation*, vol. 91, no. 2, pp. 553–565, 1993.

[21] D. A. Kujubu, S. T. Reddy, B. S. Fletcher, and H. R. Herschman, "Expression of the protein product of the prostaglandin synthase-2/TIS10 gene in mitogen-stimulated Swiss 3T3 cells," *Journal of Biological Chemistry*, vol. 268, no. 8, pp. 5425–5430, 1993.

[22] S. M. Fitzgerald and S. Flinn, "Evaluating research studies using the analysis of variance (ANOVA): issues and interpretations," *Journal of Hand Therapy*, vol. 13, no. 1, pp. 56–60, 2000.

[23] O. Braissant, F. Foufelle, C. Scotto, M. Dauça, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[24] C. Dreyer, G. Krey, H. Keller, F. Givel, G. Helftenbein, and W. Wahli, "Control of the peroxisomal β-oxidation pathway by a novel family of nuclear hormone receptors," *Cell*, vol. 68, no. 5, pp. 879–887, 1992.

[25] S. A. Kliewer, K. Umesono, D. J. Noonan, R. A. Heyman, and R. M. Evans, "Convergence of 9-*cis* retinoic acid and peroxisome proliferator signalling pathways through heterodimer formation of their receptors," *Nature*, vol. 358, no. 6389, pp. 771–774, 1992.

[26] S. A. Kliewer, B. M. Forman, B. Blumberg, et al., "Differential expression and activation of a family of murine peroxisome proliferator-activated receptors," *Proceedings of the National*

Academy of Sciences of the United States of America, vol. 91, no. 15, pp. 7355–7359, 1994.

[27] J. A. Keelan, T. A. Sato, K. W. Marvin, J. Lander, R. S. Gilmour, and M. D. Mitchell, "15-Deoxy-$\Delta^{12,14}$-prostaglandin J_2 a ligand for peroxisome proliferator-activated receptor-γ, induces apoptosis in JEG3 choriocarcinoma cells," *Biochemical and Biophysical Research Communications*, vol. 262, no. 3, pp. 579–585, 1999.

[28] X. Xin, S. Yang, J. Kowalski, and M. E. Gerritsen, "Peroxisome proliferator-activated receptor γ ligands are potent inhibitors of angiogenesis in vitro and in vivo," *Journal of Biological Chemistry*, vol. 274, no. 13, pp. 9116–9121, 1999.

[29] T. Boehm, J. Folkman, T. Browder, and M. S. O'Reilly, "Antiangiogenic therapy of experimental cancer does not induce acquired drug resistance," *Nature*, vol. 390, no. 6658, pp. 404–407, 1997.

[30] M. S. O'Reilly, T. Boehm, Y. Shing, et al., "Endostatin: an endogenous inhibitor of angiogenesis and tumor growth," *Cell*, vol. 88, no. 2, pp. 277–285, 1997.

[31] J. Drevs, I. Hofmann, H. Hugenschmidt, et al., "Effects of PTK787/ZK 222584, a specific inhibitor of vascular endothelial growth factor receptor tyrosine kinases, on primary tumor, metastasis, vessel density, and blood flow in a murine renal cell carcinoma model," *Cancer Research*, vol. 60, no. 17, pp. 4819–4824, 2000.

Hindawi Publishing Corporation
PPAR Research
Volume 2008, Article ID 359267, 13 pages
doi:10.1155/2008/359267

Review Article

Peroxisome Proliferator-Activated Receptors as Mediators of Phthalate-Induced Effects in the Male and Female Reproductive Tract: Epidemiological and Experimental Evidence

Giuseppe Latini,[1,2] Egeria Scoditti,[2,3] Alberto Verrotti,[4] Claudio De Felice,[5] and Marika Massaro[2,3]

[1] *Division of Neonatology, Perrino Hospital, 72100 Brindisi, Italy*
[2] *Clinical Physiology Institute (IFC-CNR), National Research Council of Italy, Lecce Section, 72100 Brindisi, Italy*
[3] *Laboratory of General Physiology, Department of Biological and Environmental Sciences and Technology, University of Lecce, 73100 Lecce, Italy*
[4] *Department of Medicine, Division of Pediatrics, University of Chieti, 66100 Chieti, Italy*
[5] *Neonatal Intensive Care Unit, Azienda Ospedaliera-Universitaria Senese, 53100 Siena, Italy*

Correspondence should be addressed to Giuseppe Latini, gilatini@tin.it

Received 28 May 2007; Revised 12 September 2007; Accepted 25 September 2007

Recommended by P. Froment

There is growing evidence that male as well as female reproductive function has been declining in human and wildlife populations over the last 40 years. Several factors such as lifestyle or environmental xenobiotics other than genetic factors may play a role in determining adverse effects on reproductive health. Among the environmental xenobiotics phthalates, a family of man-made pollutants are suspected to interfere with the function of the endocrine system and therefore to be endocrine disruptors. The definition of endocrine disruption is today extended to broader endocrine regulations, and includes activation of metabolic sensors, such as the peroxisome proliferator-activated receptors (PPARs). Toxicological studies have shown that phthalates can activate a subset of PPARs. Here, we analyze the epidemiological and experimental evidence linking phthalate exposure to both PPAR activation and adverse effects on male and female reproductive health.

1. INTRODUCTION

The phthalate esters are a class of water-insoluble, high-production-volume, synthetic organic chemicals used widely in a variety of industrial applications, including personal-care products (e.g., perfumes, lotions, cosmetics), paints, and mainly as plasticizers to confer flexibility and durability to polyvinyl chloride- (PVC-) based plastics and to make the plastic appropriate to different uses, including food, construction industry, medical devices, and pharmaceuticals since about the 1930s [1–4]. However, these plasticizers are not chemically bound to the plastic products, but leak out from PVC items into the environment with time and use. As a consequence, they have been found everywhere in the environment and are universally considered ubiquitous environmental contaminants. Di-(2-ethylhexyl) phthalate (DEHP) is the most abundant phthalate in the environment and mono-(2-ethylhexyl) phthalate (MEHP) is its primary metabolite [1–4]. Other important phthalates production- and applicationwise are diethyl phthalate (DEP), dibutyl phthalate (DBP), di-iso- and di-n-butyl phthalate (DiBuP, DnBuP), butyl-benzyl phthalate (BBP), di-isononylphpthalate (DiNP) and di-n-octyl phthalate (DnOP) [5]. Humans are exposed to phthalates for their whole lifetime, since intrauterine life [6–11].

The ability of these pollutants to affect human health is a major concern. In particular, evidence suggestive of harmful effects on the male reproductive system and related outcomes have gradually accumulated in recent years. In addition, there is wide demonstration that reproductive functions are altered by endocrine disrupting chemicals (EDCs), including phthalates. These chemicals have been found to

interfere with the function of the endocrine system, which is responsible for growth, sexual development, and many other essential physiological functions in both genders.

EDCs can act genomically, with agonistic or antagonistic effects on steroid receptors and may alter reproductive function and/or cause feminization by binding to oestrogen or androgen receptors. However, EDCs can also act by nongenomic mechanisms, altering steroid synthesis [12, 13].

The definition of endocrine disruption is today extended to broader endocrine regulations, and includes activation of metabolic sensors, such as a subset of nuclear hormone receptor superfamily members called peroxisome proliferator-activated receptors (PPARs).

To this regard, a large group of industrial and pharmaceutical chemicals, including phthalates, are known for their ability to provoke peroxisome proliferation, thus increasing both the size and number of peroxisomes [14]. Peroxisomes are essential organelles of eukaryotic origin, ubiquitously distributed in cells and organisms, which perform various metabolic functions (peroxide-derived respiration, beta oxidation of fatty acids, cholesterol metabolism, etc.) within the cell [15].

Many of the adaptive consequences for exposure to these pollutants are mediated by PPARs, members of the nuclear hormone receptor (NRs) superfamily of ligand-activated transcription factors. They are activated by binding of natural ligands, such as polyunsaturated fatty acids or by synthetic ligands. Three subtypes of PPARs (alpha, beta, and gamma) have been identified in different tissues, encoded by separate genes [16].

Several studies in recent years have revealed their importance in both normal physiology and in the pathology of various tissues [17, 18]. In particular, human and animal studies have demonstrated that PPARs are important in placental development [19], while they are believed to play an essential role in the adverse effects elicited by EDC [20].

The aim of this review is to explore how much evidence exists linking phthalate exposure, PPARs activation, and eventual actions of PPARs as mediators of environmental toxic substances for reproductive function in both genders.

2. ENVIRONMENTAL DISSEMINATION AND EPIDEMIOLOGICAL EVIDENCE OF PHTHALATE REPRODUCTIVE TOXICITY

Globally, more than 18 billion pounds of phthalates are used each year and well above two million tons of DEHP alone are produced annually worldwide [21]. Given their high production volume, common use, and widespread environmental contamination, humans are exposed to these compounds through ingestion, inhalation, and dermal exposures on a daily basis as testified by detection of phthalates in serum, seminal fluid, amniotic fluid, breast milk, and saliva [5, 9, 22–24]. These studies have provided evidence on the relatively high variation of phthalate exposure from day to day within individuals as well as between ethnic groups, geographic areas, and ages. In particular, general population can be exposed to DEHP to a much higher extent than previously believed and an exposure of children, twice as high as the exposure of adults with respect to their body weight, has been observed [23–26].

In particular, higher DEHP exposure has been documented in neonatal intensive-care-unit infants, because of multiple medical device-related DEHP exposure [27].

In addition, Blount et al. [28] found that women of reproductive age had significantly higher urinary levels of MBP (a reproductive and developmental toxicant in rodents) than other age/gender groups. However, in spite of the alarming wide environmental diffusion and use, studies in human populations suggesting an association between phthalate exposure and adverse reproductive health outcomes are limited yet.

To this regard, chronic occupational exposure to high levels of phthalates is associated with decreased rates of pregnancy and higher rates of miscarriage in female factory workers [29, 30]. Correspondently, higher urinary phthalate levels were observed to correlate with pregnancy complications such as anemia, toxemia, and pre-eclampsia in women living near a plastics manufacturer [31]. In addition, significantly high levels of phthalates were identified in girls with thelarche, suggesting an association between plasticizers with known estrogenic and antiandrogenic activity and the cause of premature breast development in a human female population [32].

In utero exposure to phthalates has been shown to be significantly associated with a shorter pregnancy duration [7, 8] and it has been hypothesized that phthalates may play a role in inducing and/or potentiating an intrauterine inflammatory response, a well established risk factor for prematurity [33]. Moreover, an association between phthalate exposure and endometriosis has been shown, suggesting a potential role for phthalate esters in the pathogenesis of this common cause of female infertility [34, 35]. More specifically to the male reproductive system, phthalate exposure seems to be tightly correlated to the impairment of androgen activity. For example, phthalate monoesters levels in breast milk resulted to be correlated with hormone levels in healthy boys, which were indicative of lower androgen activity and reduced Leydig cell function [36], and professional long-term exposure to phthalates has been reported to be associated with altered semen quality [37, 38] and decreased serum-free testosterone [39].

In addition, impaired testicular descent and decreased anogenital distance (AGD), the most sensitive marker of antiandrogen action in toxicological studies and a sensitive measure of prenatal antiandrogen exposure have been reported in boys whose mothers had elevated prenatal phthalate exposure [43]. All together, these findings suggest an impairment of sex hormone balance by prenatal and postnatal phthalate exposure but, although suggestive of the potentially dangerous effects of phthalate exposure on human health, they are not conclusive yet, and more epidemiologic data are needed in human populations along with a better mechanistic understanding of the phthalates activities. Although the possible mechanism of action by phthalates remains, to date, largely obscure, the use of animal models have enormously contributed to characterize the

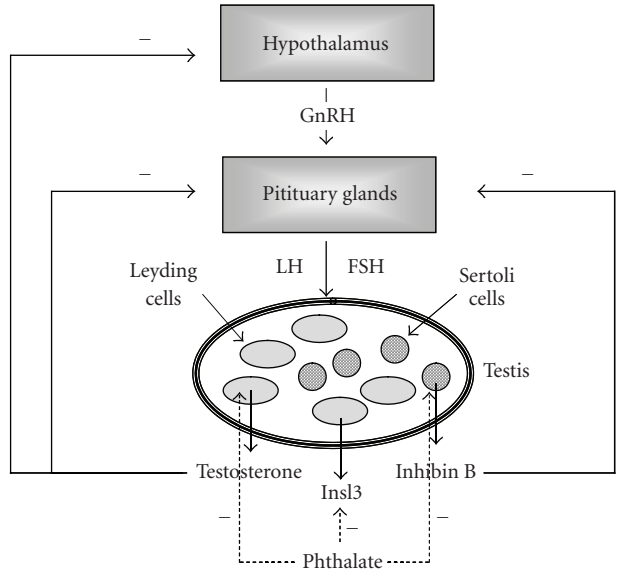


Figure 1

reproductive toxicity profiles of phthalates and to highlight the mechanisms possibly involved.

3. MALE AND FEMALE REPRODUCTIVE TRACT DEVELOPMENT: POSSIBLE INTERFERENCE SITE BY PHTHALATES

Male and female reproductive tract development is a dynamic process, requiring the production and the fine regulatory activity of sex steroid hormones: androgens, estrogens, and the progestagens [40]. Steroidal sex hormones regulate foetal developmental processes such as differentiation and sex determination. The major sites of synthesis of the sex steroids are corpus luteum for progestagens, testis for androgens, and ovaries for estrogens.

The biosynthesis of sex steroids is catalyzed by a series of enzymes that form the steroidogenic pathway [41]. This pathway causes the conversion of pregnenolone (cholesterol derivative key steroidogenic intermediate common to all classes of steroid hormones) to progesterone, the precursor for the testosterone that is formed in testis by Leydig cells through two ways: (1) Δ4-biosynthesis leads to progesterone, 17-α-hydroxyprogesterone, and androstenedione; (2) the Δ5-biosynthesis leads to 17-α-hydroxypregnenolone, dehydroepiandrosterone, and Δ5-androstendiol [41].

Androgens themselves can then be transformed to estrogens. The extent to which this biotransformation takes place depends on the expression of the various enzymes in specific tissues. The enzyme complex 19-hydroxylase-aromatase, which catalyzes the conversion of androgens to estrogens, plays a major role in this biotransformation [42].

The development of mammalian foetus into a male requires the production and action of steroid hormones, notably androgens and antimullerian hormone after testis formation, in contrast to the female development, a process largely hormone-independent [43].

Moreover, the mature reproductive function is under the regulation of the hypothalamus-pituitary-gonadal (HPG) axis. The limbic system of the brain releases specific neurotransmitters or neuropeptides that stimulate the hypothalamus to produce gonadotropin-releasing hormone (GnRH) which stimulates the pituitary gland to release specific hormones (gonadotrophins) that are transported via the blood stream to hormone-synthesizing tissues [44]. In the case of mammals, the gonadotrophins from the pituitary gland are luteinizing hormone (LH) and follicle-stimulating hormone (FSH). Under the influence of these substances, sex steroids, that is, estrogens and androgens, are released into the blood circulation from the ovaries and the testis, respectively. Negative feedback from the concentration of these gonadal steroids in the blood can lower or block the release of GnRH from the hypothalamus and of gonadotrophins at the pituitary level, thus modulating HPG axis [44].

Keeping this in mind, it might be expected that any environmental, hormonally active chemicals capable of perturbing the adequate production and action of sex hormones or the balance between estrogens and androgens during foetal life have the potential to interfere with one or more critical aspects of reproductive function (Figure 1).

4. PRE- AND POSTNATAL DEVELOPMENTAL AND REPRODUCTIVE TOXICITY BY PHTHALATES

Chronic exposure of laboratory animals to phthalates has been reported to lead to severe adverse effects, including foetal death, carcinogenesis, teratogenesis, and hepatotoxicity [45–47]. In particular, a wide range of developmental and reproductive toxicities in mammals are induced by phthalates. Phthalates can directly affect fetal and neonatal testis differentiation, inducing male rat reproductive tract malformations, as well as testicular changes remarkably similar to testicular dysgenesis syndrome (TDS) in humans [48–52].

Testicular dysgenesis, or abnormal testicular development, after in utero phthalate exposure has been shown to be

associated with abnormal function of both Sertoli and Leydig cells and abnormal sex organs development [52, 53].

Sertoli cells play a critical role in foetal testis development regulating the dynamic process of movement, organization, differentiation of all the cell types within the testis [54]. As a consequence, the abnormal function of Sertoli cells associated with phthalate exposure [52, 53] might alter the differentiation signals normally implicated in tissue morphogenesis, thus leading to many of the histological and functional anomalies observed in TDS (Figure1).

Leydig cells, the principal providers of steroid hormones in the testis, are also targeted by phthalates. To this regard, the highly conserved role of testosterone and dihydrotestosterone (DHT), in driving male reproductive tract development (masculinization) is well known. As a consequence, in rodents the whole period of male genital tract differentiation is particularly susceptible to the effects of antiandrogens, as demonstrated by in utero exposure to flutamide, (a well-known androgen receptor antagonist) and phthalates both inducing abnormalities of androgen-regulated sexual differentiation [49]. In addition, the administration of synthetic estrogens, such as diethylstilboestrol (DES), to pregnant women and rodents causes reproductive tract abnormalities in the offspring, including cryptorchidism, [55] as well as a dose-dependent reduction in the number of Sertoli cells critically involved in spermatogenesis [56]. The ability of estrogens to reduce androgen levels or expression of androgen receptor is relevant [57]. These results suggest that abnormal intrauterine hormone levels with decreased androgen production/action or increased estrogens levels may play a role in determining adverse effects on reproductive health. Correspondently, critical to the induction of phthalate testicular toxicity is the considerable reduction in fetal and postnatal testosterone levels observed after in utero exposure to phthalates at the critical window for the androgen-dependent reproductive tract development [49, 52, 53, 58]. In particular, the exposure to DEHP decreases testosterone to levels similar to those normally found in females leading to incomplete masculinization and hypospadias and cryptorchidism [58]. Thus, several phthalate esters have been shown to carry out "antiandrogenic" activity through a mechanism that is distinct from androgen-receptor antagonism, that is, targeting the Leydig cells testosterone biosynthesis machinery. In addition, genes directly associated with testosterone biosynthesis are uniformly downregulated by phthalate exposure in the fetal testis [59]. These steroidogenic genes include those involved in cholesterol handling, such as scavenger receptor class B type 1 (SR-B1) implicated in the selective cholesterol esters uptake from high density lipoproteins, steroidogenic acute regulatory protein (StAR), that mediates cholesterol transport across the mitochondrial membrane, the rate limiting enzyme in testosterone biosynthesis, that is, cholesterol side-chain cleavage enzyme (P450 scc), that converts cholesterol into pregnenolone, 3β-hydroxysteroid dehydrogenase (3βHSD), and CYP17α [59, 60]. In addition, phthalates alter the expression of genes encoding sex steroid metabolizing enzymes in the gonads and peripheral organs such as the liver. Among these, 5α-reductase, that converts testosterone to DHT, was upregulated by DEHP in the prepubertal rat testis [61]. Aside from the interference with steroid synthesis and metabolism, the induction of cryptorchidism by phthalates is mediated by the alternative mechanism acting at the initial hormone-independent phase of testicular descent. Phthalates have indeed been shown to alter the expression of insulinlike hormone 3 (Insl3) in fetal Leydig cells [62], which plays a role in guiding the testis during its first phase of transabdominal descent.

In postnatal exposure, a strong species difference in the phthalate responsiveness is evident, with some species (Syrian hamsters, e.g.,) more resistant to phthalate toxicity possibly as a consequence of an inefficient metabolic transformation of diesters to monoesters [63]. Younger animals result, in general, more sensitive than adult ones [64]. For example, Grey observed a decrease in seminiferous tubule diameter in testis and accessory sex organs (seminal vesicle and prostate) weight after phthalate exposure in 4-week-old, but not in 15-week-old rats [64]. These effects were associated with the induction of apoptosis in germ cells, likely as a consequence of an increased generation of oxidative stress and concomitant alteration of antioxidant defences by phthalate [65]. Correspondently, the FSH signalling pathway for Sertoli cell proliferation and differentiation resulted to be impaired after phthalate exposure [66, 67].

Also in postnatal and adult rats phthalates affected steroid hormone synthesis and metabolism, as indicated by decreased testosterone serum levels in male rats acutely exposed to some active phthalates and by a decreased testosterone secretion by cultured Leydig cells treated with MEHP [68]. However, contrasting results were observed by Akingbemi et al. [69] and Eagon et al. [70] in male rat chronically exposed to environmentally relevant low levels of DEHP. Increased LH and testosterone serum levels together with an increased serum estrogen likely due to impaired Leydig cell steroidogenesis and compensatory Leydig cell proliferation were observed. The modulation by phthalate of many estrogen metabolizing enzymes seems to be very complex, since it has been reported both a downregulation [71, 72] and an upregulation [73] of the aromatase gene after phthalate exposure, depending on the cell type analyzed.

Overall, the data presented here demonstrated that certain phthalates like other environmental chemicals are capable of disrupting male reproductive tract organogenesis and function when administered to laboratory animals during pregnancy and/or postnatal life, producing types of malformations and histological changes causing infertility remarkably similar to those observed in human TDS. One mechanism responsible for this effects may be the ability to disrupt the endocrine balance, that is, androgen/estrogen activities, essential for reproductive system development and homeostasis, acting as environmental antiandrogen compounds [74]. Although this raises concern towards other factors such as lifestyle that might have influenced human fertility [75].

5. THE PPAR SYSTEM AT THE CROSSROADS BETWEEN METABOLISM AND REPRODUCTION

The identification of phthalates as environmental chemicals belonging to the family of peroxisome proliferators (PP) has

shed new insight into the potential molecular mechanism of phthalate action in the reproductive system of mammals. The pleiotropic effects induced by PP including phthalates in the rodent liver are mediated by the activation of PPARs, ligand-activated transcription factors belonging to the nuclear receptor superfamily, which also includes the steroid and thyroid hormone receptors [76]. Thus far, three PPAR isoforms (α, β, or δ, and γ), encoded by separate genes, have been identified in various tissues, with PPARα predominantly expressed in the liver, PPARγ in adipose tissue, and PPARβ in a wider range of tissue [16]. Upon activation by their lipophilic ligands, PPARs regulate gene transcription by binding to PPAR response elements (PPRE) within the promoter of target genes as heterodimers with retinoic X receptors (RXR) [16, 77]. PPARs can also repress gene expression in a DNA-binding-dependent way through the recruitment of corepressors to unliganded PPARs as well as in a DNA-binding-independent manner by interfering with other nuclear signalling pathways via protein-protein interaction (leading to formation of inactive complexes) or via competition for limiting amounts of the heterodimerization partner RXR or coactivators [78]. Fatty acids and eicosanoids have been identified as natural ligands for PPARs. More potent synthetic PPAR ligands include the fibrate and thiazolidinedione drugs, clinically used as hypolipidemic and antidiabetic agents, respectively. Since the discovery of PPARs in 1990 [17], several functions have been attributed to these receptors. PPARs play critical physiological roles regulating lipid and glucose homeostasis, cellular differentiation, proliferation, and the inflammatory/immune response, with subsequent clinically relevant implication in several diseases including dyslipidemia, diabetes, cancer, atherosclerosis. PPARα has been demonstrated to play a role in regulating lipid catabolism, whereas PPARγ controls adipocyte differentiation and lipid storage [16, 77]. Although PPARβ is less well understood, it might be a mediator in the control of brain lipid metabolism, fatty acid-induced adipogenesis, and atherogenic inflammation [77]. Given the extensive crosstalk between PPARs and other transcription factors and signalling events regulating energy balance, differentiation and other significant physiological processes in many tissues, the involvement of environmental chemicals in the PPAR system may potentially result in pathophysiologically relevant consequences for human health.

The role of PPARα in PP-induced hepatic proliferative responses was established by the development of PPARα-deficient mice by Lee et al. [79]. In contrast to wild-type control animals, PPARα homozygous-deficient mice do not exhibit hepatic peroxisomal proliferation in response to treatment with PP. Aside from modest changes in lipid profile and weight, PPARα-deficient mice are otherwise phenotypically normal [80]. Thus, the major hepatic effects of PP, including hepatocarcinogenic effects, are mediated by PPARα-dependent gene transcription and signalling events. The response to PP seems to be species-specific, with rats and mice being quite sensitive to them and humans, guinea pigs, and other species being refractory [80]. Remarkably, the hepatotoxic effects of PP are lost in humans due to the lower level of PPARα expression in human liver than in rodent one [81] and to species-specific responsiveness of PPARα [82].

Before focusing on the potential involvement of PPARs in the reproductive effects of phthalate, it would be useful to consider PPAR expression pattern in the reproductive system, since the potential PPAR-mediated effects of phthalates depend on tissue distribution of the PPAR isoforms and the PPAR-responsive genes in each tissue. All PPAR isoforms are expressed in the central nervous system and in reproductive tissues, such as gonads (testis and ovary), uterus, prostate, mammary gland, pituitary gland [83]. In the testis, both somatic and germ cells express PPAR isoforms: PPARα and β are expressed in Leydig cells and cells of seminiferous tubule (Sertoli cells and germ cells) [60, 84], while PPARγ seems to be only detectable in Sertoli cells, although weak PPARγ expression in germ cells has recently been reported [85]. All PPAR isoforms have been detected in the ovary [84]. PPARγ is the predominant isoform expressed in the granulosa cells and preovulatory follicles, but its expression falls after the LH surge [86]. In addition, PPARγ is less strongly expressed in the techal cells and in corpus luteum where it increases after ovulation [86]. However, in the absence of fertilization or embryo implantation, PPARγ expression decreases as a result of corpus luteum regression [87]. Finally, PPARγ is expressed in uterine tissue, blastocyst and, together with PPARα and β, in gestational tissues [88, 89].

The physiological role of PPARs in the reproductive tissues is not completely understood but while, on one hand, PPARα-null mice remain viable and fertile [79], on the other hand, PPARβ deletion impairs fertility [90] and PPARγ-null mutation is even embryonically lethal [91]. Indeed, recent findings suggested putative important roles for PPARs in reproductive system: the ability of PPARs to regulate energy balance may represent a potential molecular link between reproductive function and glucose and lipid metabolism. It has been shown that PPARα, whose expression is upregulated by FSH in cultured seminiferous tubules [92], may affect spermatozoa fertility by promoting lipid storage mobilization and modifying phospholipid composition. PPARβ seems to play an important role in embryo implantation as showed by its strong upregulation during the decidualization process and the appearance of placental malformations in PPARβ-null mice [90]. Finally, several lines of evidence suggest that PPARγ is critically involved in follicular development, ovulation, maintenance of corpus luteum during pregnancy, and maturation and function of placenta [83].

6. MECHANISM OF PHTHALATE ESTER REPRODUCTIVE TOXICITY: POTENTIAL ROLE OF PPARS

The involvement of phthalate-PPAR interactions in the reproductive biology alteration derives from recent findings demonstrating that phthalates are able to activate PPARα and PPARγ isoforms. Metabolic conversion of diesters to the hydrolytic monoesters seems to be essential to obtain PPAR activation and toxicological effects [93]. Indeed, hepatic peroxisomal proliferation and the associated hepatocarcinogenic response induced in rodents by DEPH are mediated by its

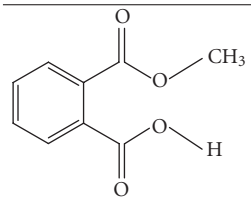
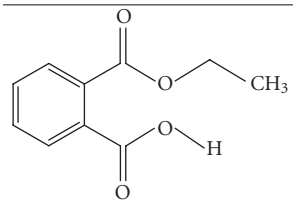
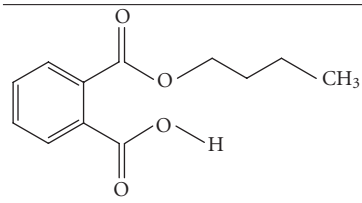
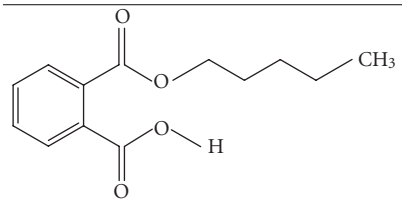
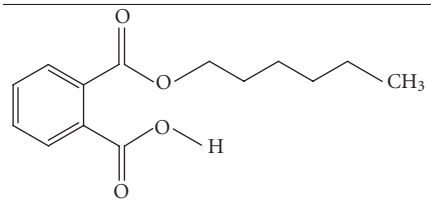
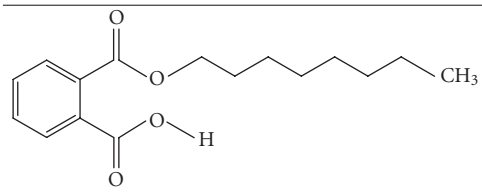
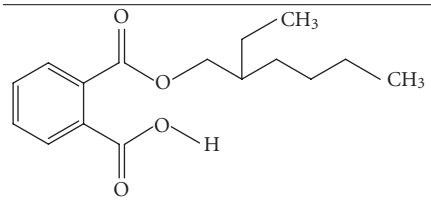
bioactive metabolite MEHP [94], which is able to activate both human and rodent PPARα and PPARγ in in vitro transactivation assay [95]. In addition to MEHP, other structurally diverse phthalate monoesters, most notably monobenzyl phthalate (mBzP), the primary metabolite of butyl benzyl phthalate (BBP), and mono-*sec*-butyl phthalate (MBuP) are capable of activating both human PPAR isoforms and target genes [93, 96] with potential implication for human health as these reproductive toxicants have been detected in human urine samples at exceptionally higher levels than MEHP itself [28]. However, it has been recently found that the diesters DEHP and BBP themselves were able to activate PPARα and PPARγ to some extent, although it was likely attributable to low level of esterases activity in the cell model used [96]. Interestingly, analyses of structure-activity relationship have found that PP in general are amphipathic carboxilates thus resembling natural PPAR ligands such as long-chain saturated and unsaturated fatty acids [97]. The carboxyl moiety of monoesters is critical for ligand activity: for example, some DEHP metabolites, such as MEHP and 2-ethylhexanoic acid, are more potent PPAR activators than 2-etylhexanol metabolite [98]. The rank order for phthalate activation of mouse and human PPARα and PPARγ agrees with the relative ability of phthalate esters to induce the classical PPAR responses, that are liver peroxisomal proliferation in rodents for PPARα and adipocyte differentiation for PPARγ [93, 99]. Indeed, it has been found that esters with long and branch-side chain are more potent PPAR activators than those containing short-chains or straight-chains. As regards PPARβ, only phthalate monoesters with longer and branch-side chains can activate this isoform but at a concentration higher than that required for activation of PPARα and PPARγ [100]. Importantly, human PPARs are less sensitive to phthalate monoesters than the corresponding mouse receptors [93]. Since the activation of PPAR assessed by transactivation assay might result from indirect events, such as endogenous production of a metabolite from the test compound or release of endogenous ligand, these compounds had to be tested further for direct binding to the PPARs. Although activation of PPARs by some phthalates may occur indirectly through release of endogenous lipid activators (fatty acids) from carrier proteins, notably fatty acid binding protein (FABP) or through a yet unidentified intermediate factor [101], recent findings reported that some relevant monoester phthalates are able of directly binding PPARα and PPARγ receptors [96]. Consistent with their ability to activate PPARs in transactivation assay, BBP and DBP weakly interact with both isoforms.

Although in most cases there has been found a correlation between PPAR activation by phthalate monoesters and reproductive toxicity by the corresponding diesters, there exist also findings weakening the assumption of a general obligatory role for PPARs in mediating phthalate-induced reproductive effects. For example, while di-isononyl phthalate (DINP) is a weak reproductive toxicant [102], its monoester metabolite MINP is a moderately strong PPAR activator [100]. In addition, DBP is a strong reproductive toxicant through its proximal metabolite MBP [103] and induces hepatotoxicity in rodents via PPARα [104], although MBP only weakly activates PPARs in transactivation assay [93]. One possible interpretation of these discordant results may be the involvement of an indirect mechanism of PPAR activation mediated by an unknown endogenous metabolite activator, not necessarily detectable by using transactivation assay.

Only a few studies in PPARα-null mice directly determined the role of PPAR in phthalate-induced male developmental and reproductive toxicities. The study by Peters et al. [105] showed that prenatal exposure to DEHP caused developmental malformations in both wild-type and PPARα knockout mice, thus suggesting a PPARα-independent mechanism. However, it is difficult to draw any conclusion about the role of PPARα in phthalate reproductive toxicity since the intrauterine administration of DEHP occurred before the critical period of reproductive tract differentiation. Another important animal study demonstrated that intrauterine DEHP-treated PPARα-deficient mice, predominantly normal at earlier time point, developed delayed testicular, renal and developmental toxicities, but not liver toxicity, compared to wild types [104], thus first confirming the early observation by Lee et al. about the PPARα dependence of liver response and, more importantly, indicating that DEHP may induce reproductive toxicity through both PPARα-dependent and -independent mechanism. Another study found that the administration of DEHP resulted in milder testis lesions and higher testosterone levels in PPARα-null mice than in wild-type mice [106]. In contrast, the PPARα-independent reproductive toxicity observed by Ward et al. may conceivably be mediated by other PPAR isoforms, such as PPARβ and PPARγ, or by a nonreceptor-mediated organ-specific mechanism. Unfortunately, till now no studies have been performed in PPARβ-null mice, and the toxicological impacts of phthalates that activate PPARγ are unknown. Determining a role for PPARγ in phthalate-induced reproductive toxicity requires testis-specific-knockout mice as PPARγ deletion results in the death of the embryo [91]. Notably, both PPARα and PPARγ are responsive to DEHP in vitro and are translocated to the nucleus in primary Sertoli cells after incubation of these cells with phthalate esters [107, 108]. Given the key role played by Sertoli cells in driving testis morphogenesis, it may be therefore hypothesized that the impairment of this cell type by MEHP contributed to the observed testicular toxicity.

The potential of PPARs to mediate the endocrine disruption activity by phthalates is also suggested from the finding that a few genes involved in steroid biosynthesis and metabolism are directly regulated by PPARs. MEHP activates both PPARα and PPARγ in cultured rat granulosa cells which cause a complete inhibition of aromatase gene expression [109–111]. In addition, the estradiol metabolizing enzyme 17β-HSD IV has been shown to be induced by MEHP in the liver and granulosa cells through a PPARα-dependent mechanism [112]. Therefore, both decreased estradiol synthesis and increased estradiol metabolism contribute to suppressed serum estradiol levels observed after DEHP in vivo exposure and to the subsequent female reproductive toxicity [71, 72, 113]. Finally, the induction by DEHP of FABP expression in the liver via PPARα [114] and in granulosa cells via both PPARα and PPARγ [115] may play important role in

Table 1: Structures and related name of the most common phthalate monoesters. Diesters of *o*-phthalic acid are quickly metabolized in vivo to their active metabolites, the monesters. The length and structure of the side chain are important for toxicity.

Chemical structure	Systematic name	Abbreviation
	Monomethyl phthalate	MMP
	Monoethyl phthalate	MEP
	Monobutyl phthalate	MBP
	Monopentyl phthalate	MPP
	Monohexyl phthalate	MHP
	Monopropyl phthalate	MPrP
	Mono-(2-ethylhexyl) phthalate	MEPH

the mechanism of phthalate effect on steroid hormones since FABP functions as an intracellular gateway for PPAR agonists [116] and as a donor of potential fatty acid ligands of PPARs [101].

Taking into account the specific tissue distribution and the physiological roles of PPAR isoforms, one could speculate upon some phthalate effects in mammals. It is known that cells exposed to PP undergo oxidative stress possibly due to PPARα-mediated activation of metabolizing enzymes in the liver and associated with the hepatic toxicity of DEHP [117]. Genes involved in oxidative stress response have been shown to be upregulated in the liver by DEHP exposure [118]. In

addition, the induction of xenobiotic metabolizing enzymes by PPARα after DEHP exposure could increase the susceptibility to other environmental toxicants requiring metabolic activation [118]. PPARγ is a prototypic adipocyte differentiation regulator [119] and activation of PPARγ by phthalates in other tissue and subsequent alteration of differentiation pathways may be implicated in phthalate teratogenic effects. In addition, PPARγ may be part of the LH-induced luteinization in the ovary since its activation causes aromatase downregulation, this event being essential for the postovulatory phenotype [120]. The activation of PPARγ by phthalates in the preovulatory follicle prevented the estradiol increase necessary for stimulating the ovulatory surge of LH and prematurely induces follicle differentiation to a postovulatory phenotype [113].

7. DEVELOPMENTAL AND REPRODUCTIVE TOXICITY OF PHTHALATES IN FEMALE ANIMAL MODELS

The above-mentioned epidemiological evidence suggesting adverse consequences for female reproductive function [30, 31] stimulated more in depth studies in animal models on the issue. Besides causing developmental toxicity, including high incidence of foetus death and malformations and reduced foetal body weight, DEHP administration to pregnant rodents decreased embryo implantation and increased resorptions [121, 122]. These effects were mimicked by other phthalate esters thus representing both male and female reproductive toxicants in rodents [123].

The administration of phthalate esters, including DEHP and its metabolite MEHP, to adult female rats caused an increase in the estrous cycle length and dysovulation, associated with polycystic ovaries, and decreased serum levels of estradiol [71]. These functional changes were associated with morphological alteration of the preovulatory follicle, the site of estradiol production, where granulosa cells were smaller in DEHP-treated mice than in control rats, and incapable of mounting an ovulatory surge of LH. Regarding the molecular mechanism by which DEHP/MEHP suppressed estradiol production in the granulosa cells, it has been found that MEHP inhibits FSH-stimulated cAMP accumulation and progesterone production in granulosa cells [124]. When the progesterone precursor pregnenolone is added to granulosa cell cultures treated with MEHP, the inhibition of progesterone production is reversed [125]. However MEHP did not decrease the expression of P450 scc [126], the major regulatory site of progesterone production by cAMP which converts cholesterol to pregnenolone [127]. In addition to reducing progesterone production at a site prior to pregnenolone, MEHP also reduces estradiol production by affecting aromatase gene expression, the rate-limiting enzyme that converts testosterone to estradiol. Aromatase is stimulated by FSH-mediated pathways and techal androgens. Androgens are the substrates for aromatization to estradiol in granulosa cells [128]. Thus, MEHP is able to decrease estradiol production independent of its effect on FSH–cAMP and decreases aromatase activity without acting as a direct enzyme inhibitor [72]. Furthermore, the induction by both DEHP and DBP of the estradiol metabolizing enzyme 17β-HSD IV in the liver and granulosa cells [112, 129] contributes to explain the suppressed serum estradiol levels after DEHP exposure and the significant increase in serum levels of estrone, the primary metabolite of estradiol, observed in DBP-treated rats [71].

Overall, these findings underline once again that phthalate toxicant effects on female reproductive system is attributable to an interference with the complex and tightly regulated machinery involved in steroid synthesis and metabolism. Notably, the pathways leading to production of ovarian hormones are similar in rodent models and humans, and using the rodent model to determine the mechanism of action of MEHP will aid in understanding how exposure to this chemical may affect ovarian function in women.

8. CONCLUSIONS

Phthalates are environmental contaminants with significant human exposures. These chemicals may act as EDCs and alter reproductive function and/or cause feminization raising concern about the potential health hazards posed by such exposures. The adverse effects of phthalates have been chiefly studied in animal models, while their potential toxicity to humans together with the possible involvement of PPARs in mediating these effects on the reproductive health has to be more properly evaluated. Pre- and/or perinatal periods appear to be critical windows of exposure, because of their high sensitivity to hormonal dysregulation by EDCs. Thus, the acquisition of more detailed data on human exposure during these time periods is essential. It has been proposed that impairment of reproductive development and function in both genders by phthalates relates to abnormal steroid biosynthesis and metabolism and seems to be at least in part mediated by the activation of the PPAR signalling pathway. Molecular basis for the adverse health effects proposed to be associated with human phthalate exposure have to be elucidated. Finally, analysis of the effects of phthalate exposures on gonadotropin and steroid hormone levels should form part of overall risk assessment in human populations.

REFERENCES

[1] ATDSR, "Toxicological Profile for Diethylphthalate," Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry: Atlanta, 1995.

[2] ATDSR, "Toxicological Profile for di-*n*-octyl phthalate," Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry: Atlanta, 1997.

[3] ATDSR, "Toxicological Profile for di-*n*-butyl phthalate," Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry: Atlanta, 2001.

[4] ATDSR, "Toxicological Profile for di-(2-ethylhexyl)phthalate (DEHP)," Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry: Atlanta, 2002.

[5] H. M. Koch, B. Rossbach, H. Drexler, and J. Angerer, "Internal exposure of the general population to DEHP and other

phthalates—determination of secondary and primary phthalate monoester metabolites in urine," *Environmental Research*, vol. 93, no. 2, pp. 177–185, 2003.

[6] J. J. Adibi, F. P. Perera, W. Jedrychowski, et al., "Prenatal exposures to phthalates among women in New York and Krakow, Poland," *Environmental Health Perspectives*, vol. 111, no. 14, pp. 1719–1722, 2003.

[7] G. Latini, C. De Felice, G. Presta, et al., "In utero exposure to di-(2-ethylhexyl)phthalate and duration of human pregnancy," *Environmental Health Perspectives*, vol. 111, no. 14, pp. 1783–1785, 2003.

[8] G. Latini, C. de Felice, G. Presta, et al., "Exposure to di-(2-ethylhexyl)phthalate in humans during pregnancy: a preliminary report," *Biology of the Neonate*, vol. 83, no. 1, pp. 22–24, 2003.

[9] M. J. Silva, J. A. Reidy, A. R. Herbert, J. L. Preau Jr., L. L. Needham, and A. M. Calafat, "Detection of phthalate metabolites in human amniotic fluid," *Bulletin of Environmental Contamination and Toxicology*, vol. 72, no. 6, pp. 1226–1231, 2004.

[10] S. H. Swan, "Prenatal phthalate exposure and anogenital distance in male infants," *Environmental Health Perspectives*, vol. 114, no. 2, pp. A88–A89, 2006.

[11] S. H. Swan, K. M. Main, F. Liu, et al., "Decrease in anogenital distance among male infants with prenatal phthalate exposure," *Environmental Health Perspectives*, vol. 113, no. 8, pp. 1056–1061, 2005.

[12] R. Kavlock, K. Boekelheide, R. Chapin, et al., "NTP center for the evaluation of risks to human reproduction: phthalates expert panel report on the reproductive and developmental toxicity of di-*n*-octyl phthalate," *Reproductive Toxicology*, vol. 16, no. 5, pp. 721–734, 2002.

[13] R. H. Waring and R. M. Harris, "Endocrine disrupters: a human risk?" *Molecular and Cellular Endocrinology*, vol. 244, no. 1-2, pp. 2–9, 2005.

[14] J. Ehrmann Jr., N. Vavrusová, Y. Collan, and Z. Kolár, "Peroxisome proliferator-activated receptors (PPARs) in health and disease," *Biomedical Papers of the Medical Faculty of the University Palacky*, vol. 146, no. 2, pp. 11–14, 2002.

[15] H. F. Tabak, D. Hoepfner, A. V. D. Zand, H. J. Geuze, I. Braakman, and M. A. Huynen, "Formation of peroxisomes: present and past," *Biochimica et Biophysica Acta*, vol. 1763, no. 12, pp. 1647–1654, 2006.

[16] T. Lemberger, B. Desvergne, and W. Wahli, "Peroxisome proliferator-activated receptors: a nuclear receptor signaling pathway in lipid physiology," *Annual Review of Cell and Developmental Biology*, vol. 12, pp. 335–363, 1996.

[17] I. Issemann and S. Green, "Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators," *Nature*, vol. 347, no. 6294, pp. 645–650, 1990.

[18] S. Yu and J. K. Reddy, "Transcription coactivators for peroxisome proliferator-activated receptors," *Biochimica et Biophysica Acta*, vol. 1771, no. 8, pp. 936–951, 2007.

[19] T. Fournier, V. Tsatsaris, K. Handschuh, and D. Evain-Brion, "PPARs and the placenta," *Placenta*, vol. 28, no. 2-3, pp. 65–76, 2007.

[20] J. N. Feige, L. Gelman, D. Rossi, et al., "The endocrine disruptor monoethyl-hexyl-phthalate is a selective peroxisome proliferator-activated receptor γ modulator that promotes adipogenesis," *Journal of Biological Chemistry*, vol. 282, no. 26, pp. 19152–19166, 2007.

[21] B. C. Blount, K. E. Milgram, M. J. Silva, et al., "Quantitative detection of eight phthalate metabolites in human urine using HPLC-APCI-MS/MS," *Analytical Chemistry*, vol. 72, no. 17, pp. 4127–4134, 2000.

[22] A. M. Calafat, L. L. Needham, M. J. Silva, and G. Lambert, "Exposure to di-(2-ethylhexyl)phthalate among premature neonates in a neonatal intensive care unit," *Pediatrics*, vol. 113, no. 5, pp. e429–434, 2004.

[23] M. J. Silva, D. B. Barr, J. A. Reidy, et al., "Urinary levels of seven phthalate metabolites in the U.S. population from the National Health and Nutrition Examination Survey (NHANES) 1999-2000," *Environmental Health Perspectives*, vol. 112, no. 3, pp. 331–338, 2004.

[24] M. J. Silva, A. R. Slakman, J. A. Reidy, et al., "Analysis of human urine for fifteen phthalate metabolites using automated solid-phase extraction," *Journal of Chromatography B*, vol. 805, no. 1, pp. 161–167, 2004.

[25] H. M. Koch, H. M. Bolt, and J. Angerer, "Di-(2-ethylhexyl)phthalate (DEHP) metabolites in human urine and serum after a single oral dose of deuterium-labelled DEHP," *Archives of Toxicology*, vol. 78, no. 3, pp. 123–130, 2004.

[26] H. M. Koch, H. Drexler, and J. Angerer, "Internal exposure of nursery-school children and their parents and teachers to di-(2-ethylhexyl)phthalate (DEHP)," *International Journal of Hygiene and Environmental Health*, vol. 207, no. 1, pp. 15–22, 2004.

[27] R. Green, R. Hauser, A. M. Calafat, et al., "Use of di-(2-ethylhexyl)phthalate-containing medical products and urinary levels of mono-(2-ethylhexyl)phthalate in neonatal intensive care unit infants," *Environmental Health Perspectives*, vol. 113, no. 9, pp. 1222–1225, 2005.

[28] B. C. Blount, M. J. Silva, S. P. Caudill, et al., "Levels of seven urinary phthalate metabolites in a human reference population," *Environmental Health Perspectives*, vol. 108, no. 10, pp. 979–982, 2000.

[29] L. E. Milkov, M. V. Aldyreva, T. B. Popova, et al., "Health status of workers exposed to phthalate plasticizers in the manufacture of artificial leather and films based on PVC resins," *Environmental Health Perspectives*, vol. 3, pp. 175–178, 1973.

[30] M. V. Aldyreva, T. S. Klimova, A. S. Izyumova, and L. A. Timofievskaya, "The effect of phthalate plasticizers on the generative function," *Gigiena Truda I Professional'nye Zabolevaniia*, vol. 19, pp. 25–29, 1975.

[31] S. Tabacova, R. Little, and L. Balabaeva, "Maternal exposure to phthalates and complications of pregnancy," *Epidemiology*, vol. 10, p. 127, 1999.

[32] I. Colón, D. Caro, C. J. Bourdony, and O. Rosario, "Identification of phthalate esters in the serum of young Puerto Rican girls with premature breast development," *Environmental Health Perspectives*, vol. 108, no. 9, pp. 895–900, 2000.

[33] L. F. Gonçalves, T. Chaiworapongsa, and R. Romero, "Intrauterine infection and prematurity," *Mental Retardation and Developmental Disabilities Research Reviews*, vol. 8, no. 1, pp. 3–13, 2002.

[34] L. Cobellis, G. Latini, C. De Felice, et al., "High plasma concentrations of di-(2-ethylhexyl)phthalate in women with endometriosis," *Human Reproduction*, vol. 18, no. 7, pp. 1512–1515, 2003.

[35] B. S. Reddy, R. Rozati, B. V. R. Reddy, and N. V. V. S. S. Raman, "Association of phthalate esters with endometriosis in Indian women," *BJOG: An International Journal of Obstetrics and Gynaecology*, vol. 113, no. 5, pp. 515–520, 2006.

[36] G. Lottrup, A.-M. Andersson, H. Leffers, et al., "Possible impact of phthalates on infant reproductive health," *International Journal of Andrology*, vol. 29, no. 1, pp. 172–180, 2006.

[37] S. M. Duty, M. J. Silva, D. B. Barr, et al., "Phthalate exposure and human parameters," *Epidemiology*, vol. 14, no. 3, pp. 269–277, 2003.

[38] R. Rozati, P. P. Reddy, P. Reddanna, and R. Mujtaba, "Role of environmental estrogens in the deterioration of male factor fertility," *Fertility and Sterility*, vol. 78, no. 6, pp. 1187–1194, 2002.

[39] G. Pan, T. Hanaoka, M. Yoshimura, et al., "Decreased serum free testosterone in workers exposed to high levels of di-*n*-butyl phthalate (DBP) and di-2-ethylhexyl phthalate (DEHP): a cross-sectional study in China," *Environmental Health Perspectives*, vol. 114, no. 11, pp. 1643–1648, 2006.

[40] A. Jost, "Action of various sex and related steroids on the growth and sexual differentiation of fetuses," *Acta Endocrinologica. Supplementum*, vol. 50, pp. 119–123, 1960.

[41] R. V. Brooks, "Androgens," *Clinical Endocrinology & Metabolism*, vol. 4, pp. 503–520, 1975.

[42] K. Suhara, K. Ohashi, K. Takeda, and M. Katagiri, "P-450(11β)-dependent conversion of androgen to estrogen, the aromatase reaction," *Biochemical and Biophysical Research Communications*, vol. 140, no. 2, pp. 530–535, 1986.

[43] J. D. Wilson, "Sexual differentiation," *Annual Review of Physiology*, vol. 40, pp. 279–306, 1978.

[44] C. H. Rodgers, "Neuroendocrine mechanisms responsible for gonadotropin release," *The Journal of Reproductive Medicine*, vol. 14, no. 1, pp. 1–7, 1975.

[45] B. G. Lake, "Mechanisms of hepatocarcinogenicity of peroxisome-proliferating drugs and chemicals," *Annual Review of Pharmacology and Toxicology*, vol. 35, pp. 483–507, 1995.

[46] G. Latini, M. Massaro, and C. De Felice, "Prenatal exposure to phthalates and intrauterine inflammation: a unifying hypothesis," *Toxicological Sciences*, vol. 85, no. 1, p. 743, 2005.

[47] E. A. Lock, A. M. Mitchell, and C. R. Elcombe, "Biochemical mechanisms of induction of hepatic peroxisome proliferation," *Annual Review of Pharmacology and Toxicology*, vol. 29, pp. 145–163, 1989.

[48] E. Mylchreest, R. C. Cattley, and P. M. D. Foster, "Male reproductive tract malformations in rats following gestational and lactational exposure to di-(*n*-butyl) phthalate: an antiandrogenic mechanism?" *Toxicological Sciences*, vol. 43, no. 1, pp. 47–60, 1998.

[49] E. Mylchreest, M. Sar, R. C. Cattley, and P. M. D. Foster, "Disruption of androgen-regulated male reproductive development by di-(*n*-butyl) phthalate during late gestation in rats is different from flutamide," *Toxicology and Applied Pharmacology*, vol. 156, no. 2, pp. 81–95, 1999.

[50] E. Mylchreest, M. Sar, D. G. Wallace, and P. M. D. Foster, "Fetal testosterone insufficiency and abnormal proliferation of Leydig cells and gonocytes in rats exposed to di-(*n*-butyl) phthalate," *Reproductive Toxicology*, vol. 16, no. 1, pp. 19–28, 2002.

[51] E. Mylchreest, D. G. Wallace, R. C. Cattley, and P. M. D. Foster, "Dose-dependent alterations in androgen-regulated male reproductive development in rats exposed to di-(*n*-butyl) phthalate during late gestation," *Toxicological Sciences*, vol. 55, no. 1, pp. 143–151, 2000.

[52] P. M. D. Foster, "Mode of action: impaired fetal Leydig cell function—effects on male reproductive development produced by certain phthalate esters," *Critical Reviews in Toxicology*, vol. 35, no. 8-9, pp. 713–719, 2005.

[53] P. M. D. Foster, "Disruption of reproductive development in male rat offspring following in utero exposure to phthalate esters," *International Journal of Andrology*, vol. 29, no. 1, pp. 140–147, 2006.

[54] P. Koopman, "Gonad development: signals for sex," *Current Biology*, vol. 11, no. 12, pp. R481–R483, 2001.

[55] R. J. Stillman, "In utero exposure to diethylstilbestrol: adverse effects on the reproductive tract and reproductive performance in male and female offspring," *American Journal of Obstetrics and Gynecology*, vol. 142, no. 7, pp. 905–921, 1982.

[56] R. M. Sharpe, C. McKinnell, C. Kivlin, and J. S. Fisher, "Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood," *Reproduction*, vol. 125, no. 6, pp. 769–784, 2003.

[57] A. Rivas, J. S. Fisher, C. McKinnell, N. Atanassova, and R. M. Sharpe, "Induction of reproductive tract developmental abnormalities in the male rat by lowering androgen production or action in combination with a low dose of diethylstilbestrol: evidence for importance of the androgen-estrogen balance," *Endocrinology*, vol. 143, no. 12, pp. 4797–4808, 2002.

[58] L. G. Parks, J. S. Ostby, C. R. Lambright, et al., "The plasticizer di-ethylhexyl phthalate induces malformations by decreasing fetal testosterone synthesis during sexual differentiation in the male rat," *Toxicological Sciences*, vol. 58, no. 2, pp. 339–349, 2000.

[59] N. J. Barlow, S. L. Phillips, D. G. Wallace, M. Sar, K. W. Gaido, and P. M. D. Foster, "Quantitative changes in gene expression in fetal rat testes following exposure to di-(*n*-butyl) phthalate," *Toxicological Sciences*, vol. 73, no. 2, pp. 431–441, 2003.

[60] V. D. Shultz, S. Phillips, M. Sar, P. M. D. Foster, and K. W. Gaido, "Altered gene profiles in fetal rat testes after in utero exposure to di-(*n*-butyl) phthalate," *Toxicological Sciences*, vol. 64, no. 2, pp. 233–242, 2001.

[61] H.-S. Kim, K. Saito, M. Ishizuka, A. Kazusaka, and S. Fujita, "Short period exposure to di-(2-ethylhexyl)phthalate regulates testosterone metabolism in testis of prepubertal rats," *Archives of Toxicology*, vol. 77, no. 8, pp. 446–451, 2003.

[62] V. S. Wilson, C. Lambright, J. Furr, et al., "Phthalate ester-induced gubernacular lesions are associated with reduced insl3 gene expression in the fetal rat testis," *Toxicology Letters*, vol. 146, no. 3, pp. 207–215, 2004.

[63] T. J. B. Gray, I. R. Rowland, P. M. D. Foster, and S. D. Gangolli, "Species differences in the testicular toxicity of phthalate esters," *Toxicology Letters*, vol. 11, no. 1-2, pp. 141–147, 1982.

[64] T. J. B. Gray and S. D. Gangolli, "Aspects of the testicular toxicity of phthalate esters," *Environmental Health Perspectives*, vol. 65, pp. 229–235, 1986.

[65] A. Agarwal, R. A. Saleh, and M. A. Bedaiwy, "Role of reactive oxygen species in the pathophysiology of human reproduction," *Fertility and Sterility*, vol. 79, no. 4, pp. 829–843, 2003.

[66] L.-H. Li, W. F. Jester Jr., A. L. Laslett, and J. M. Orth, "A single dose of di-(2-ethylhexyl)phthalate in neonatal rats alters gonocytes, reduces Sertoli cell proliferation, and decreases cyclin D2 expression," *Toxicology and Applied Pharmacology*, vol. 166, no. 3, pp. 222–229, 2000.

[67] L.-H. Li, W. F. Jester Jr., and J. M. Orth, "Effects of relatively low levels of mono-(2-ethylhexyl)phthalate on cocultured Sertoli cells and gonocytes from neonatal rats," *Toxicology and Applied Pharmacology*, vol. 153, no. 2, pp. 258–265, 1998.

[68] H. B. Jones, D. A. Garside, R. Liu, and J. C. Roberts, "The influence of phthalate esters on Leydig cell structure and function in vitro and in vivo," *Experimental and Molecular Pathology*, vol. 58, no. 3, pp. 179–193, 1993.

[69] B. T. Akingbemi, R. T. Youker, C. M. Sottas, et al., "Modulation of rat Leydig cell steroidogenic function by di-(2-ethylhexyl)phthalate," *Biology of Reproduction*, vol. 65, no. 4, pp. 1252–1259, 2001.

[70] P. K. Eagon, N. Chandar, M. J. Epley, M. S. Elm, E. P. Brady, and K. N. Rao, "Di-(2-ethylhexyl)phthalate-induced changes in liver estrogen metabolism and hyperplasia," *International Journal of Cancer*, vol. 58, no. 5, pp. 736–743, 1994.

[71] B. J. Davis, R. R. Maronpot, and J. J. Heindel, "Di-(2-ethylhexyl)phthalate suppresses estradiol and ovulation in cycling rats," *Toxicology and Applied Pharmacology*, vol. 128, no. 2, pp. 216–223, 1994.

[72] B. J. Davis, R. Weaver, L. J. Gaines, and J. J. Heindel, "Mono-(2-ethylhexyl)phthalate suppresses estradiol production independent of FSH-cAMP stimulation in rat granulosa cells," *Toxicology and Applied Pharmacology*, vol. 128, no. 2, pp. 224–228, 1994.

[73] B. T. Akingbemi, R. Ge, G. R. Klinefelter, B. R. Zirkin, and M. P. Hardy, "Phthalate-induced Leydig cell hyperplasia is associated with multiple endocrine disturbances," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 101, no. 3, pp. 775–780, 2004.

[74] J. S. Fisher, "Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome," *Reproduction*, vol. 127, no. 3, pp. 305–315, 2004.

[75] R. M. Sharpe and S. Franks, "Environment, lifestyle and infertility—an inter-generational issue," *Nature Cell Biology*, vol. 4, no. s1, pp. S33–S40, 2002.

[76] B. Desvergne and W. Wahli, "Peroxisome proliferator-activated receptor: nuclear control of metabolism," *Endocrine Reviews*, vol. 20, no. 5, pp. 649–688, 1999.

[77] V. Bocher, G. Chinetti, J. C. Fruchart, and B. Staels, "Role of the peroxisome proliferator-activated receptors (PPARS) in the regulation of lipids and inflammation control," *Journal of Biomedicine and Biotechnology*, vol. 196, no. 1, pp. 47–52, 2002.

[78] G. Chinetti, J.-C. Fruchart, and B. Staels, "Peroxisome proliferator-activated receptors (PPARs): nuclear receptors at the crossroads between lipid metabolism and inflammation," *Inflammation Research*, vol. 49, no. 10, pp. 497–505, 2000.

[79] S. S. Lee, T. Pineau, J. Drago, et al., "Targeted disruption of the α isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators," *Molecular and Cellular Biology*, vol. 15, pp. 3012–3022, 1995.

[80] F. J. Gonzalez, J. M. Peters, and R. C. Cattley, "Mechanism of action of the nongenotoxic peroxisome proliferators: role of the peroxisome proliferator-activated receptor," *Journal of the National Cancer Institute*, vol. 90, no. 22, pp. 1702–1709, 1998.

[81] C. N. Palmer, M. H. Hsu, K. J. Griffin, J. L. Raucy, and E. F. Johnson, "Peroxisome proliferator activated receptor-α expression in human liver," *Molecular Pharmacology*, vol. 53, pp. 14–22, 1998.

[82] H. Keller, P. R. Devchand, M. Perroud, and W. Wahli, "PPARα structure-function relationships derived from species-specific differences in responsiveness to hypolipidemic agents," *Biological Chemistry*, vol. 378, no. 7, pp. 651–655, 1997.

[83] P. Froment, F. Gizard, D. Defever, B. Staels, J. Dupont, and P. Monget, "Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition," *Journal of Endocrinology*, vol. 189, no. 2, pp. 199–209, 2006.

[84] O. Braissant, F. Foufelle, C. Scotto, M. Dauca, and W. Wahli, "Differential expression of peroxisome proliferator-activated receptors (PPARs): tissue distribution of PPAR-α, -β, and -γ in the adult rat," *Endocrinology*, vol. 137, no. 1, pp. 354–366, 1996.

[85] K. Thomas, D. Sung, X. Chen, R. Gibbs, J. McCarrey, and W. Walker, "Developmental patterns of PPAR/RXR gene expression during spermatogenesis," in *Society for the Study of Reproduction (SSR '05)*, Quebec City, Canada, July 2005.

[86] C. M. Komar, O. Braissant, W. Wahli, and T. E. Curry Jr., "Expression and localization of PPARs in the rat ovary during follicular development and the periovulatory period," *Endocrinology*, vol. 142, no. 11, pp. 4831–4838, 2001.

[87] T. Viergutz, B. Loehrke, R. Poehland, F. Becker, and W. Kanitz, "Relationship between different stages of the corpus luteum and the expression of the peroxisome proliferator-activated receptor γ protein in bovine large lutein cells," *Journal of Reproduction and Fertility*, vol. 118, no. 1, pp. 153–161, 2000.

[88] M. Mohan, S. Ryder, P. L. Claypool, R. D. Geisert, and J. R. Malayer, "Analysis of gene expression in the bovine blastocyst produced in vitro using suppression-subtractive hybridization," *Biology of Reproduction*, vol. 67, no. 2, pp. 447–453, 2002.

[89] E. B. E. Berry, R. Eykholt, R. J. A. Helliwell, R. S. Gilmour, M. D. Mitchell, and K. W. Marvin, "Peroxisome proliferator-activated receptor isoform expression changes in human gestational tissues with labor at term," *Molecular Pharmacology*, vol. 64, no. 6, pp. 1586–1590, 2003.

[90] Y. Barak, D. Liao, W. He, et al., "Effects of peroxisome proliferator-activated receptor δ on placentation, adiposity, and colorectal cancer," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 99, no. 1, pp. 303–308, 2002.

[91] Y. Barak, M. C. Nelson, E. S. Ong, et al., "PPARγ is required for placental, cardiac, and adipose tissue development," *Molecular Cell*, vol. 4, no. 4, pp. 585–595, 1999.

[92] R. Schultz, W. Yan, J. Toppari, A. Völkl, J.-Å. Gustafsson, and M. Pelto-Huikko, "Expression of peroxisome proliferator-activated receptor α messenger ribonucleic acid and protein in human and rat testis," *Endocrinology*, vol. 140, no. 7, pp. 2968–2975, 1999.

[93] C. H. Hurst and D. J. Waxman, "Activation of PPARα and PPARγ by environmental phthalate monoesters," *Toxicological Sciences*, vol. 74, no. 2, pp. 297–308, 2003.

[94] P. W. Albro and S. R. Lavenhar, "Metabolism of di-(2-ethylhexyl)phthalate," *Drug Metabolism Reviews*, vol. 21, pp. 13–34, 1989.

[95] E. K. Maloney and D. J. Waxman, "Trans-activation of PPARα and PPARγ by structurally diverse environmental chemicals," *Toxicology and Applied Pharmacology*, vol. 161, no. 2, pp. 209–218, 1999.

[96] P. J. Lapinskas, S. Brown, L. M. Leesnitzer, et al., "Role of PPARα in mediating the effects of phthalates and metabolites in the liver," *Toxicology*, vol. 207, no. 1, pp. 149–163, 2005.

[97] S. A. Kliewer, S. S. Sundseth, S. A. Jones, et al., "Fatty acids and eicosanoids regulate gene expression through direct interactions with peroxisome proliferator-activated receptors α and γ," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 94, no. 9, pp. 4318–4323, 1997.

[98] Y. Keith, M. C. Cornu, P. M. Canning, J. M. D. Foster, J. C. Lhuguenot, and C. R. Elcombe, "Peroxisome proliferation due to di-(2-ethylhexyl) adipate, 2-ethylhexanol and

2-ethylhexanoic acid," *Archives of Toxicology*, vol. 66, no. 5, pp. 321–326, 1992.

[99] E. D. Barber, B. D. Astill, E. J. Moran, et al., "Peroxisome induction studies on seven phthalate esters," *Toxicology and Industrial Health*, vol. 3, no. 2, pp. 7–24, 1987.

[100] M. T. Bility, J. T. Thompson, R. H. McKee, et al., "Activation of mouse and human peroxisome proliferator-activated receptors (PPARs) by phthalate monoesters," *Toxicological Sciences*, vol. 82, no. 1, pp. 170–182, 2004.

[101] D. J. Luebker, K. J. Hansen, N. M. Bass, J. L. Butenhoff, and A. M. Seacat, "Interactions of flurochemicals with rat liver fatty acid-binding protein," *Toxicology*, vol. 176, no. 3, pp. 175–185, 2002.

[102] L. E. Gray Jr., J. Ostby, J. Furr, M. Price, D. N. R. Veeramachaneni, and L. Parks, "Perinatal exposure to the phthalates DEHP, BBP, and DINP, but not DEP, DMP, or DOTP, alters sexual differentiation of the male rat," *Toxicological Sciences*, vol. 58, no. 2, pp. 350–365, 2000.

[103] P. M. D. Foster, R. C. Cattley, and E. Mylchreest, "Effects of di-*n*-butyl phthalate (DBP) on male reproductive development in the rat: implications for human risk assessment," *Food and Chemical Toxicology*, vol. 38, supplement 1, pp. S97–S99, 2000.

[104] J. M. Ward, J. M. Peters, C. M. Perella, and F. J. Gonzalez, "Receptor and nonreceptor-mediated organ-specific toxicity of di-(2-ethylhexyl)phthalate (DEHP) in peroxisome proliferator-activated receptor alpha-null mice," *Toxicology and Pathology*, vol. 26, no. 2, pp. 240–246, 1998.

[105] J. M. Peters, M. W. Taubeneck, C. L. Keen, and F. J. Gonzalez, "Di-(2-ethylhexyl)phthalate induces a functional zinc deficiency during pregnancy and teratogenesis that is independent of peroxisome proliferator-activated receptor-α," *Teratology*, vol. 56, no. 5, pp. 311–316, 1997.

[106] M. Gazouli, Z.-X. Yao, N. Boujrad, J. C. Corton, M. Culty, and V. Papadopoulos, "Effect of peroxisome proliferators on Leydig cell peripheral-type benzodiazepine receptor gene expression, hormone-stimulated cholesterol transport, and steroidogenesis: role of the peroxisome proliferator-activator receptor α," *Endocrinology*, vol. 143, no. 7, pp. 2571–2583, 2002.

[107] J. M. Dufour, M.-N. Vo, N. Bhattacharya, J. Okita, R. Okita, and K. H. Kim, "Peroxisome proliferators disrupt retinoic acid receptor alpha signaling in the testis," *Biology of Reproduction*, vol. 68, no. 4, pp. 1215–1224, 2003.

[108] N. Bhattacharya, J. M. Dufour, M.-N. Vo, J. Okita, R. Okita, and H. K. Kwan, "Differential effects of phthalates on the testis and the liver," *Biology of Reproduction*, vol. 72, no. 3, pp. 745–754, 2005.

[109] Y.-M. Mu, T. Yanase, Y. Nishi, R. Takayanagi, K. Goto, and H. Nawata, "Combined treatment with specific ligands for PPARγ:RXR nuclear receptor system markedly inhibits the expression of cytochrome P450arom in human granulosa cancer cells," *Molecular and Cellular Endocrinology*, vol. 181, no. 1-2, pp. 239–248, 2001.

[110] T. Lovekamp-Swan and C. L. Chaffin, "The peroxisome proliferator-activated receptor γ ligand troglitazone induces apoptosis and p53 in rat granulosa cells," *Molecular and Cellular Endocrinology*, vol. 233, no. 1-2, pp. 15–24, 2005.

[111] J. C. Corton and P. J. Lapinskas, "Peroxisome proliferator-activated receptors: mediators of phthalate ester-induced effects in the male reproductive tract?" *Toxicological Sciences*, vol. 83, no. 1, pp. 4–17, 2005.

[112] J. C. Corton, C. Bocos, E. S. Moreno, A. Merritt, R. C. Cattley, and J.-Å. Gustafsson, "Peroxisome proliferators alter the expression of estrogen-metabolizing enzymes," *Biochimie*, vol. 79, no. 2-3, pp. 151–162, 1997.

[113] T. Lovekamp-Swan and B. J. Davis, "Mechanisms of phthalate ester toxicity in the female reproductive system," *Environmental Health Perspectives*, vol. 111, no. 2, pp. 139–145, 2003.

[114] H. Poirier, I. Niot, M.-C. Monnot, et al., "Differential involvement of peroxisome-proliferator-activated receptors α and δ in fibrate and fatty-acid-mediated inductions of the gene encoding liver fatty-acid-binding protein in the liver and the small intestine," *Biochemical Journal*, vol. 355, no. 2, pp. 481–488, 2001.

[115] T. Lovekamp-Swan, A. M. Jetten, and B. J. Davis, "Dual activation of PPARα and PPARγ by mono-(2-ethylhexyl)phthalate in rat ovarian granulosa cells," *Molecular and Cellular Endocrinology*, vol. 201, no. 1-2, pp. 133–141, 2003.

[116] C. Wolfrum, C. M. Borrmann, T. Börchers, and F. Spener, "Fatty acids and hypolipidemic drugs regulate peroxisome proliferator-activated receptors α- and γ-mediated gene expression via liver fatty acid binding protein: a signaling path to the nucleus," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 98, no. 5, pp. 2323–2328, 2001.

[117] J. K. Reddy and M. S. Rao, "Oxidative DNA damage caused by persistent peroxisome proliferation: its role in hepatocarcinogenesis," *Mutation Research*, vol. 214, no. 1, pp. 63–68, 1989.

[118] J. S. Wong and S. S. Gill, "Gene expression changes induced in mouse liver by di-(2-ethylhexyl)phthalate," *Toxicology and Applied Pharmacology*, vol. 185, no. 3, pp. 180–196, 2002.

[119] P. Tontonoz, E. Hu, R. A. Graves, A. I. Budavari, and B. M. Spiegelman, "mPPARγ2: tissue-specific regulator of an adipocyte enhancer," *Genes and Development*, vol. 8, no. 10, pp. 1224–1234, 1994.

[120] S. L. Fitzpatrick, D. L. Carlone, R. L. Robker, and J. S. Richards, "Expression of aromatase in the ovary: down-regulation of mRNA by the ovulatory luteinizing hormone surge," *Steroids*, vol. 62, no. 1, pp. 197–206, 1997.

[121] A. F. Kaul, P. F. Souney, and R. Osathanondh, "A review of possible toxicity of di-2-ethylhexylphthalate (DEHP) in plastic intravenous containers: effects on reproduction," *Drug Intelligence and Clinical Pharmacy*, vol. 16, no. 9, pp. 689–692, 1982.

[122] I. Tomita, Y. Nakamura, Y. Yagi, and K. Tutikawa, "Fetotoxic effects of mono-2-ethylhexyl phthalate (MEHP) in mice," *Environmental Health Perspectives*, vol. 65, pp. 249–254, 1986.

[123] J. J. Heindel and C. J. Powell, "Phthalate ester effects on rat Sertoli cell function in vitro: effects of phthalate side chain and age of animal," *Toxicology and Applied Pharmacology*, vol. 115, no. 1, pp. 116–123, 1992.

[124] K. A. Treinen, W. C. Dodson, and J. J. Heindel, "Inhibition of FSH-stimulated cAMP accumulation and progesterone production by mono-(2-ethylhexyl)phthalate in rat granulosa cell cultures," *Toxicology and Applied Pharmacology*, vol. 106, no. 2, pp. 334–340, 1990.

[125] K. A. Treinen and J. J. Heindel, "Evidence that MEHP inhibits rat granulosa cell function by a protein kinase C-independent mechanism," *Reproductive Toxicology*, vol. 6, no. 2, pp. 143–148, 1992.

[126] T. N. Lovekamp and B. J. Davis, "Mono-(2-ethylhexyl)phthalate suppresses aromatase transcript levels and estradiol production in cultured rat granulosa cells," *Toxicology and Applied Pharmacology*, vol. 172, no. 3, pp. 217–224, 2001.

[127] A. J. Hsueh, E. Y. Adashi, P. B. Jones, and T. H. Welsh Jr., "Hormonal regulation of the differentiation of cultured ovarian granulosa cells," *Endocrine Reviews*, vol. 5, pp. 76–127, 1984.

[128] J. S. Richards, "Maturation of ovarian follicles: actions and interactions of pituitary and ovarian hormones on follicular cell differentiation," *Physiological Reviews*, vol. 60, no. 1, pp. 51–89, 1980.

[129] L.-Q. Fan, R. C. Cattley, and J. C. Corton, "Tissue-specific induction of 17β-hydroxysteroid dehydrogenase type IV by peroxisome proliferator chemicals is dependent on the peroxisome proliferator-activated receptor α," *Journal of Endocrinology*, vol. 158, no. 2, pp. 237–246, 1998.