Multiple roles of the prostaglandin D<sub>2</sub> signaling pathway in reproduction

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Abstract

Prostaglandins signaling molecules are involved in numerous physiological processes. They are produced by several enzyme-limited reactions upon fatty acids, which are catalyzed by two cyclooxygenases and prostaglandin synthases. In particular, the prostaglandins E<sub>2</sub> (PGE<sub>2</sub>), D<sub>2</sub> (PGD<sub>2</sub>), and F<sub>2</sub> (PGF<sub>2a</sub>) have been shown to be involved in female reproductive mechanisms. Furthermore, widespread expression of lipocalin- and hematopoietic-PGD<sub>2</sub> synthases in the male reproductive tract supports the purported roles of PGD<sub>2</sub> in the development of both embryonic and adult testes, sperm maturation, and spermatogenesis. In this review, we summarize the putative roles of PGD<sub>2</sub> signaling and the roles of both PGD<sub>2</sub> synthases in testicular formation and function. We review the data reporting the involvement of PGD<sub>2</sub> signaling in the differentiation of Sertoli and germ cells of the embryonic testis. Furthermore, we discuss the roles of lipocalin-PGD<sub>2</sub> synthase in steroidogenesis and spermatogenesis, in terms of lipid molecule transport and PGD<sub>2</sub> production. Finally, we discuss the hypothesis that PGD<sub>2</sub> signaling may be affected in certain reproductive diseases, such as infertility, cryptorchidism, and testicular cancer.


Introduction

Prostaglandins (PGs) derived from polyunsaturated fatty acids belong to the superfamily of eicosanoids. The eicosanoid cascade starts with the activation of phospholipases A<sub>2</sub> and C that release arachidonic acid from the cellular membrane. Arachidonic acid is oxidized and then reduced by the enzymes cyclooxygenases 1 and 2 (COX1 and COX2, also referred to as prostaglandin endoperoxidase H synthase 1 and 2 (PTGS1 and PTGS2)), to be converted into PGG<sub>2</sub> and PGH<sub>2</sub>. The COXs are key enzymes in PG biosynthesis and differ in their expression levels and tissue distribution; COX1 is constitutively expressed, whereas expression of COX2 is induced (Simmons et al. 2004). PGH<sub>2</sub>, the unstable reaction intermediate, is then converted into either PGD<sub>2</sub>, PGE<sub>2</sub>, PGF<sub>2a</sub> or prostacyclin (PGI<sub>2</sub>) and thromboxane A<sub>2</sub> (TXA<sub>2</sub>), by the action of specific terminal PG synthases; prostaglandin D synthase (PGDS), prostaglandin E synthase (PGES), prostaglandin F synthase (PGFS) or prostacyclin synthase (PGIS), or thromboxane synthase (TXS) respectively (Fig. 1 and Table 1) (Cha et al. 2006). PGs are rapidly inactivated by oxidation by the NAD+dependent 15-hydroxyprostaglandin dehydrogenase (15-PGDH) (Fincham & Camp 1983). PGs are involved in the cardiovascular, gastrointestinal, genitourinary, endocrine, respiratory, immune, and nervous systems (Hata & Breyer 2004). These molecules act locally in an autocrine and/or paracrine manner and their actions are complex, not least because, given the structural similarity of these molecules and their receptors, PGs may have synergistic or antagonistic effects upon the same physiological processes (Woodward et al. 2011, Tootle 2013).

PGD<sub>2</sub> is actively produced in many organs, and is the most abundant prostanooid in the CNS (Urade & Hayashi 2000a) and in the respiratory tract and airways of asthmatic patients (Oguma et al. 2008). PGD<sub>2</sub> has essential roles in various physiological processes (Matsuoka et al. 2000, Kobayashi & Narumiya 2002, Qu et al. 2006, Huang et al. 2007, Taniguchi et al. 2007, Oguma et al. 2008, Gao et al. 2009, Nieves & Garza 2014), and particularly in several steps of the reproduction function (this function will be discussed further in the following paragraphs). Also, PGD<sub>2</sub> together with the prostaglandins PGE<sub>2</sub> and PGI<sub>2</sub>, in conjunction with other mediators such as histamine, are involved in the inflammation process (Hata & Breyer 2004, Herlong & Scott 2006). Hematopoietic PGDS (H-PGDS) is the key enzyme in the synthesis of PGD<sub>2</sub> in the immune system and mast cells (Urade & Hayashi 2000a, Kanaoka & Urade 2003). Furthermore, the resolution of inflammation is accompanied by a shift from the biosynthesis
of PGES to that of lipocalin PGDS (L-PGDS) (Schuligoi et al. 2005, Nicolaou et al. 2014).

PGD\textsubscript{2} synthesis and its regulation

PGD\textsubscript{2} synthesis is regulated by the functional and differential coupling of COX1 and COX2 enzymes with both PGDS and PGES (Ueno et al. 2005). The expression of COX2 is induced by growth factors such as pro-inflammatory cytokines (IL1, TNF\textalpha), and inhibited by glucocorticoids and other anti-inflammatory cytokines (IL4 and IL10) (Loftin et al. 2002, Morita 2002). In particular, COX2 is induced by interleukin-1 in the testes of infertile men, stimulating the production of PGD\textsubscript{2} and PGF\textsubscript{2\alpha} (Matzkin et al. 2010). Testosterone induces COX2 expression and PGF\textsubscript{2\alpha} production in hamster Leydig cells through a nonclassical mechanism involving MAPK signaling (Matzkin et al. 2009). Silencing of Ptgs2 through G9a- and EZH2-mediated histone methylation and DNA methylation of its promoter region has also been reported (Coward et al. 2014). On the other hand, the nonsteroidal anti-inflammatory drugs (NSAIDS) inhibit COX enzymatic activities through noncompetitive (Aspirin) or competitive binding to the active site (Cha et al. 2006).

The synthesis of PGD\textsubscript{2} is under the specific control of two PGDS, the L-PGDS (or PTGDS), and the H-PGDS (or PTGDS2) (Urade & Eguchi 2002). Originally identified in the rat brain, L-PGDS, whose function is independent of the tripeptide glutathione, is part of the lipocalin protein superfamily, the members of which are secreted into the extracellular space (Urade & Hayaishi 2000a). This enzyme is produced in the CNS (brain, spinal cord, dorsal root ganglia), in the male genitalia (testes, epididymides, prostate) (Fouchecourt et al. 2002), and in the heart (Eguchi et al. 1997). It has been suggested that L-PGDS has dual functions. Associated with the endoplasmic reticulum and the outer nuclear membrane, it catalyzes the final step in PGD\textsubscript{2} synthesis from a common PG precursor. Secondly, as L-PGDS is secreted in many fluids (cerebrospinal fluid, seminal plasma, ascites, serum, urine, and amniotic fluid), it has been proposed to have a role in binding and transporting small hydrophobic ligands such as retinol, \beta-lactoglobulin, bile pigments, and thyroid hormones (Urade & Hayaishi 2000b, Fouchecourt et al. 2002).

The expression of L-Pgds is also under the control of many regulatory factors, protein kinase C (PKC) (Fujimori et al. 2005), estrogens (Mong et al. 2003), IL1\beta, RasGRP4

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**Figure 1** Pathway of prostanoid biosynthesis and signaling. Arachidonic acid is metabolized by the action of cyclooxygenase (COX) first to prostaglandin endoperoxide (PGG\textsubscript{2}) and then to PGH\textsubscript{2}, which is subsequently converted to various prostaglandins (PG\textsubscript{D}{\textsubscript{2}}, PG\textsubscript{J}{\textsubscript{2}}, PG\textsubscript{E}{\textsubscript{2}}, PG\textsubscript{F}{\textsubscript{2\alpha}}, PG\textsubscript{I}{\textsubscript{2}}, and thromboxane A\textsubscript{2} (TXA\textsubscript{2}) by respective synthases. COX, cyclooxygenase; PGES, prostaglandin E synthase; PGDS, prostaglandin D synthase; PGFS, prostaglandin F synthase; PGIS, prostacyclin synthase; TXS, thromboxane synthase. Individual prostaglandin interacts with specific members of the subfamily of the G protein-coupled receptor (GCPR) superfamily of seven transmembrane-spanning proteins, DP\textsubscript{1}, DP\textsubscript{2}, EP\textsubscript{1–4}, FP, IP, and TP. Then, these receptors activate different transduction (cAMP/ Ca\textsuperscript{2+}\textsubscript{I/3}) and signaling pathways.
(Li et al. 2003), each being highly cell-type specific. PGD₂ itself induces L-Pgds expression through binding of the Nrf2 factor on the L-Pgds promoter region in macrophages (Kim et al. 2013). In vitro primary cultures of rat Sertoli cells also show the activation of L-PGDS protein expression after treatment with progesterone or retinoic acid (RA) (Samy et al. 2000). RA strongly induces the accumulation of L-PGDS mRNA in human 3AO ovarian cancer cells, leading to the inhibition of their proliferation (Su et al. 2003). Furthermore, in the embryonic male gonad, L-PGDS expression is initiated and maintained by the testis differentiating factor SOX9 (Moniot et al. 2009) (see below).

Originally identified in the rat spleen, H-PGDS is a member of the class of glutathione-S-transferase enzymes, which are cytosolic and play a role in detoxification. Bivalent Ca²⁺ and Mg²⁺ ions increase the activity of H-PGDS; however, only Mg²⁺ increases its affinity for glutathione (Inoue et al. 2003). Despite the high homology of the primary sequence in different species, the tissue expression profile is highly variable. Expression is high in the peripheral tissue, spleen, thymus, bone marrow, gastrointestinal tract, and oviduct of rats (Kanaoka & Urade 2003). In the mouse, expression is predominant in the skin, oviduct (Kanaoka et al. 2000), and granulosa cells of the postnatal and adult ovary (Farhat et al. 2011). However, in humans, expression is found in the placenta, lung, fetal liver, heart, brain, mastocytes, lymphocytes, Th2 cells, and antigen-presenting cells (Kanaoka et al. 2000, Tanaka et al. 2000).

PGD₂ is dehydrated in vitro and in vivo by a nonezymatic process to produce PGs of the J series, PGJ₂, and 15-deoxy-12,14-PGJ₂ (Shibata et al. 2002). These PGD₂ metabolites can also influence diverse cellular functions. In particular, H-PGDS was shown to control the onset and resolution of acute inflammation through PGD₂ and 15-d PGJ₂ (Rajakariar et al. 2007).

Table 1  Abbreviations.

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Name</th>
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<tr>
<td>COX1 or PTGS1</td>
<td>Cyclooxygenase 1 or prostaglandin G/H synthase 1</td>
</tr>
<tr>
<td>COX2 or PTGS2</td>
<td>Cyclooxygenase 2</td>
</tr>
<tr>
<td>DP₁</td>
<td>Prostaglandin D₂ receptor 1</td>
</tr>
<tr>
<td>DP₂ or CRTH₂</td>
<td>Prostaglandin D₂ receptor 2</td>
</tr>
<tr>
<td>H-PGDS or PTGDS2</td>
<td>Hematopoietic-type prostaglandin D₂ synthase</td>
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<tr>
<td>HQL-79</td>
<td>4-benzhydryloxy-1-[3-(1H-tetrazol-5-yl)-propyl]-piperidine</td>
</tr>
<tr>
<td>L-PGDS or PTGDS</td>
<td>Lipocalin-type prostaglandin D₂ synthase</td>
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<tr>
<td>NSAIDS</td>
<td>Nonsteroidal anti-inflammatory drugs</td>
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<tr>
<td>PG</td>
<td>Prostaglandins</td>
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<tr>
<td>PGD₂</td>
<td>Prostaglandin D₂</td>
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<tr>
<td>PGDS</td>
<td>Prostaglandin D synthase</td>
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<td>PGE₅</td>
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<tr>
<td>PGF₂α</td>
<td>Prostaglandin F₂α</td>
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<td>PGFS</td>
<td>Prostaglandin F synthase</td>
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<tr>
<td>PGJ₂</td>
<td>Prostaglandin J₂</td>
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<tr>
<td>PGI₂</td>
<td>Prostaglandin I₂ or prostacyclin</td>
</tr>
<tr>
<td>TXS</td>
<td>Thromboxane A synthase</td>
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<tr>
<td>COX1 or PTGS1</td>
<td>Cyclooxygenase 1 or prostaglandin G/H synthase 1</td>
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**PGD₂ signal transduction**

PGs are secreted and activate nine different receptors (Fig. 1): DP₁ and DP₂ or chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH₂) for PGD₂, EP₁₋₄ for PGE₂, FP for PGF₂α, IP for PGI₂, and TP for TxA₂ (Breyer et al. 2001). PG receptors are categorized as three clusters of a distinct subfamily of the G protein-coupled receptor (GPCR) superfamily of seven transmembrane-spanning proteins (Coleman et al. 1994). The only exception is DP₂, a member of the chemoattractant receptor subgroup. These receptors transduce different signals via the production of second messenger cAMP or IP3/diacylglycerol/Ca²⁺ (Woodward et al. 2011) (Fig. 1).

Thus, PGD₂ may bind to two receptors, the DP₁ receptor (Boie et al. 1995) and/or the DP₂ receptor CRTH₂ (Hirai et al. 2001). Activation of the DP₁ receptor, coupled to a Gαs protein, induces the production of the second messenger cAMP, which stimulates protein kinase A (PKA) and also induces an influx of Ca²⁺ (Boie et al. 1995). The activation of CRTH₂ or DP₂ receptors coupled to a Gαi protein inhibits cAMP production (Hirai et al. 2001) and induces intracellular Ca²⁺ mobilization caused by the production of inositol triphosphate (Woodward et al. 2011). On the other hand, the PGD₂ metabolite 15d-PGI₂ was identified as a ligand for the peroxisome proliferator-activated receptor gamma (PPARγ), a member of the nuclear receptor family (Forman et al. 1995) and for DP₂.

**PGD₂ and reproduction in adult gonads**

**Female reproduction**

Few studies have evaluated the involvement of PGD₂ in female reproduction. H-PGDS and both DP₁ and CRTH₂ receptors are expressed in the placenta and L-PGDS is present in amniotic fluid, indicating a role in the regulation of placental communication (Lumsden et al. 1986, Saito et al. 2002). H-Pgds mRNA was localized in the granulosa cells from primary to pre-ovulatory follicles of the mouse adult ovary (Farhat et al. 2011). In this tissue, H-PGDS-induced PGD₂ interferes with FSH signaling through increased Fshr and Lhcgr (LHR) receptor expression, leading to the activation of steroidogenic Cyp11a1 and Star gene expression, and subsequently to progesterone secretion. Furthermore, H-PGDS-induced PGD₂ is involved in the regulation of follicular growth through inhibition of granulosa cell proliferation in growing follicles (Farhat et al. 2011).

However, numerous roles of other PGs, PGE₂, PGF₂α, and PGI₂ have been highlighted in different stages of blastocyst implantation: vascular permeabilization, stromal decidualization, blastocyst growth and development, leukocyte recruitment, embryo transport, trophoblast invasion, and extracellular matrix remodeling (Salleh 2014). The respective contribution of these PGs...
in female reproduction was highlighted through the analysis of the Cox1 and/or Cox2-knockout mice (Morita 2002). In particular, Cox2 gene-deficient mice have defective ovulation, fertilization, and implantation mechanisms (Loftin et al. 2001). This phenotype was mimicked in Ep2−/− receptor mice, demonstrating the involvement of PGE2 in these processes. COX2, PGE2, and Ptger2, synthesized in follicular cumulus cells in response to gonadotropins, induce the follicle and oocyte maturation necessary for fertilization and ovulation (Kobayashi & Narumiya 2002). Furthermore, mice with targeted disruption of the Cox1 gene have delayed parturition resulting in neonatal death, demonstrating the role of Cox1 for the initiation of labor (Gross et al. 1998). PGF2α, which is highly expressed in the uterus, is involved in this process via the FP receptor. Moreover, PGF2α expression in the corpus luteum of the ovary is also involved in the apoptosis of these cells in the absence of gestation (Hasumoto et al. 1997).

**Male reproduction**

In the male, Cox1−/− and Cox2−/− mouse models do not exhibit perturbed reproductive processes. However, L-PGDS is widely expressed in the testis and caput epididymis of bull and mouse models (Gerena et al. 2000a,b). L-PGDS is detected in bovine and human seminal plasma (Gerena et al. 1998, Tokugawa et al. 1998) and its concentration is lower in oligozoospermic than in normozoospermic men (Tokugawa et al. 1998), suggesting that this protein plays a role in both the development and maturation of sperm and emphasizes the role of L-PGDS in spermatogenesis. L-Pgds mRNA expression is found mainly in Leydig cells (Baker & O’Shaughnessy 2001), prospermatogonia, and SOX9-expressing Sertoli cells (Moniot et al. 2009) of the adult mouse testis. In rat, L-PGDS was detected in the Sertoli and germ cells of the adult testis (Samy et al. 2000). In humans, L-PGDS, H-PGDS, and DP1 receptor are also expressed in the interstitial compartments of testes with normal and impaired spermatogenesis (Schell et al. 2007). L-PGDS and H-PGDS are expressed in Leydig cells and mast cells, respectively, along with COX2, in testes with impaired spermatogenesis. COX1 and COX2 are shown to be absent in normal human testes, whereas they are highly expressed in testicular cancer, and act to induce the growth of testicular cancer cells (Hase et al. 2003). The expression of COX2 in testicular biopsies from patients with mixed atrophy is correlated with H-PGDS expression in the mast cells of these testes (Welter et al. 2011). The major function of L-PGDS in spermatogenesis may be related with its role in the supply of retinooids, thyroid hormones, and essential fatty acids for the development of germ cells in the seminiferous tubules and maturing spermatzoa in the epididymides (Urade & Hayaishi 2000a). However, the role of L-PGDS in male reproduction remains unclear (Leone et al. 2002).

H-PGDS expression in the male gonad is not well documented. The expression was detected in the Leydig cells and mast cells of the testes of human patients with impaired spermatogenesis (Schell et al. 2007) and in the germ cells of murine testes (personal data not shown); however, its role in reproduction is unknown.

15-Deoxy PGJ2, a metabolite of PGD2 influences the expression of differentiation markers (SMC, smooth muscle actin) and the contractibility of the human peritubular cells of the testes (Schell et al. 2010) and thus, may be involved in infertility (Welter et al. 2013). COX2 mRNA expression was greatly increased in experimental cryptorchid testes, when compared with contralateral testes. Furthermore, in the spermatocytes of the cryptorchid testes the COX2 protein was specifically upregulated, thus protecting germ cells against apoptosis and disturbance of spermatogenesis (Kubota et al. 2011).

Furthermore, PGD2 induced testosterone production in Leydig cells isolated from hamster testes (Schell et al. 2007). On the other hand, COX2 activity was shown to reduce steroidogenesis by decreasing Star gene expression in MA-10 mouse Leydig cells (Wang et al. 2003). However, the role of PGD2 in the steroidogenesis process is still unclear because other reports using organotypic cultures of adult human gonads did not find a link between the effect of analgesics on PG synthesis and inhibition of testosterone production (Albert et al. 2013).

**PGD2 and the formation of embryonic male gonads**

**PGD2 signaling components are expressed in embryonic testes**

Amongst the male-enriched bands, identified by representational difference analysis (RDA) at embryonic stage E12.5, the gene encoding for L-PGDS was identified (Adams & McLaren 2002). The expression of L-Pgds mRNA in developing urogenital ridges was first detected in the Sertoli cells and prospermatogonia of late E11.5 male genital ridges (Schell et al. 2007). L-PGDS and H-PGDS are expressed in Leydig cells and mast cells, respectively, along with COX2, in testes with impaired spermatogenesis. COX1 and COX2 are shown to be absent in normal human testes, whereas they are highly expressed in testicular cancer, and act to induce the growth of testicular cancer cells (Hase et al. 2003). The expression of COX2 in testicular biopsies from patients with mixed atrophy is correlated with H-PGDS expression in the mast cells of these testes (Welter et al. 2011). The major function of L-PGDS in spermatogenesis may be related with its role in the supply of retinoids, thyroid hormones, and essential fatty acids for the development of germ cells in the seminiferous tubules and maturing spermatzoa in the epididymides (Urade & Hayaishi 2000a). However, the role of L-PGDS in male reproduction remains unclear (Leone et al. 2002).
seminiferous tubules in the embryonic gonad and neonatal testis to the interstitial compartment, particularly the Leydig cells in the adult testis (Baker & O’Shaughnessy 2001, Moniot et al. 2009).

Concerning the second PGD2-inducing H-Pgds enzyme, H-Pgds mRNA expression in both germ and somatic cells was found in E11.5–E17.5 gonads. In addition, the H-PGDS protein is also expressed in both cell types (Moniot et al. 2011, 2014). Production of PGD2 by both the somatic and germ cell lineages was confirmed, using chemical fixation of PGD2 on its production site (Bandeira-Melo et al. 2011) followed by immunofluorescence analysis, suggesting that both L-PGDS and H-PGDS enzyme capabilities are active within the embryonic gonad (Moniot et al. 2014). On the other hand, in the E13.5 male gonad, the DP1 receptor is only expressed in somatic cells whereas the DP2 is expressed in both germ cells and somatic compartments, at the mRNA and protein levels (Moniot et al. 2014).

**PGD2 signaling is involved in somatic differentiation**

In most mammals, somatic sex determination in males is initiated in undifferentiated embryonic gonads by the expression of the Sry gene, which occurs at stages E10.5–E12.5 in mice, initiating testis differentiating Sox9 expression (Sekido & Lovell-Badge 2008). The master effector gene Sox9 encodes a transcription factor that belongs to the HMG superfamily (Wagner et al. 1994). Before sex determination and before the peak of Sry expression at E11.5, SOX9 is excluded from the nucleus in the genital ridge of both sexes (Morais da Silva et al. 1996, de Santa Barbara et al. 2000), via a nuclear export signal (NES), located in its HMG domain (Gasca et al. 2002), and is retained in the cytoplasm, possibly via its interaction with microtubules (Malki et al. 2005a). Upon sex determination, the SOX9 protein is transported into the nucleus in the male gonad. PGD2 signaling via its DP1 receptor and stimulation of the cAMP pathway induce SOX9 nuclear translocation via PKA phosphorylation in NT2/D1 cells (Malki et al. 2005b) (Fig. 2). Indeed, in L-Pgds−/− gonads, SOX9 subcellular localization and testis cord formation were impaired up to E13.5, even though a variable SOX9 expression pattern and sex cord formation phenotype, ranging from normal to severely abnormal, were found (Moniot et al. 2009). Furthermore, the PGD2-producing H-PGDS enzyme is expressed in the embryonic gonad at mid E11.5 (16–17 Ts), despite L-PGDS not being expressed. Inhibition of H-Pgds enzymatic activity by the specific HQL-79 inhibitor impairs nuclear translocation of the SOX9 protein in E11.5 pre-Sertoli cells, a phenotype that was also found in H-Pgds−/− XY gonads (Moniot et al. 2011), suggesting that an initial H-PGDS-mediated PGD2 signal could participate in the SOX9 nuclear translocation necessary for the process of Sertoli cell differentiation (Fig. 2).

Moreover, PGD2 has a masculinizing effect on cultivated XX gonadal explants (ectopic testicular cord formation and expression of AMH) (Adams & McLaren 2002) through the stimulation of Sox9 gene expression (Wilhelm et al. 2005), as SOX9 can directly bind to and activate the L-Pgds promoter (Wilhelm et al. 2007). L-Pgds expression was indeed abolished in E12.0 male Sox9−/− gonads (Ck19-Cre; Sox9flox/flox mice), confirming that SOX9 is required for the initiation of L-Pgds gene expression, as L-Pgds is a direct target gene for SOX9. Moreover, ablation of Sox9 after the onset of L-Pgds expression (E13.5–E14.5 Amh-Cre; Sox9flox/flox) also induces a strong downregulation of L-Pgds expression, demonstrating the requirement for the SOX9 protein in the maintenance of L-Pgds gene expression in embryonic Sertoli cells (Moniot et al. 2009). Altogether, these data show that L-Pgds and Sox9 genes are part of a regulatory loop, initiating and maintaining L-Pgds expression and upregulating Sox9. This regulatory loop is independent on the fibroblast growth factor 9 (Fgf9)/Sox9 regulatory loop previously identified (Kim et al. 2006). Indeed, the onset of L-Pgds expression was not affected in Fgf9−/− (Moniot et al. 2009) or Fgf9 receptor R2 (FgfR2−/−) (Kim et al. 2007) mutant XY gonads and Fgf9 mRNA expression was not modified in E12.5 L-Pgds−/− gonads, confirming that both pathways do not interact genetically. However, both FGF9 and PGD2 signaling molecules cooperate to additively upregulate Sox9 expression in the Sertoli-like NT2D1 cell line (Moniot et al. 2009; Fig. 2). Many endocrine disruptors (phthalates, bisphenol) and several NSAIDS that inhibit COX activities reduce PGD2 production in the SC5 mouse Sertoli cell line and in cultured rat fetal testes (Kristensen et al. 2011a,b, 2012), leading to reduced testosterone production. However, the role of PGD2 in the onset of the steroidogenesis process remains unclear. Unlike adult human or rat testes, ex vivo exposure of embryonic human testis to paracetamol, aspirin, and indomethacin has no effect either on the production of PGD2 or on the concentration of testosterone (Mazaugh-Guittot et al. 2013).

PGD2 is also involved in the process of testicular descent in mice, because adult L-Pgds−/− mice present unilateral cryptorchidism without impaired androgen signaling, but rather a decrease in the INS3 receptor Rxfp2 mRNA expression in the gubernaculum (Philibert et al. 2013). The use of NSAIDS, which inhibit COXs enzymes, during the second trimester of pregnancy is associated with an increased risk of cryptorchidism in humans (Jensen et al. 2010, Kristensen et al. 2011a); however, the nature of the PG(s) involved in this phenotype is unknown.

**PGD2 signaling is involved in germ line differentiation**

The differentiated Sertoli cells will then influence the germ cell lineage to differentiate (Svingen & Koopman 2013).
In mice, primordial germ cells (PGCs) colonize the genital ridge at around E10.5 and continue proliferating until E13.5 (McLaren 2000). At this time, in the developing ovary, germ cells enter prophase of the first meiotic division after the upregulation of the pre-meiotic gene Stra8 (Ewen & Koopman 2010). In contrast, in the testis, germ cells stop proliferating and fully enter the G0/G1 phase of the cell cycle by E15.5 (Western et al. 2008); meanwhile, pluripotent marker expression is repressed (Western et al. 2010) and male germ cell markers such as Nanos2 are upregulated, which actively inhibits meiosis entry and thus contributes to the differentiation of the germline (Suzuki & Saga 2008). Male germ cells remain quiescent until shortly after birth, at which point they resume mitosis and then initiate meiosis around 8 days post partum (dpp) (Ewen & Koopman 2010).

**Figure 2** Model for the role of PGD2 signaling in Sertoli cell and germ cell differentiation in the male embryonic gonad. Upon sex determination around E11.5, the Sertoli cell differentiating factor SOX9 is transported into the nucleus in the male gonad. PGD2 signaling via its DP1 receptor and stimulation of the cAMP pathway induces SOX9 nuclear translocation via protein kinase A (PKA) phosphorylation. PGD2 signaling through L-Pgds is part of a regulatory loop between L-Pgds and Sox9 genes, which acts independently of the FGF9/SOX9 loop. Later around E13.5, PGD2 signaling, which is produced by both the Sertoli cell and germ cells, is involved in the germline differentiation, through its DP2 receptor. PGD2 is involved in the activation of the male germ cell marker Nanos2, inhibiting meiosis through the repression of Stra8; PGD2 is also involved in the activation of the cell cycle inhibitor p21 expression and the repression of Cyclins E expression, and in the repression of the pluripotent markers expression Sox2, Pou5f1, and Nanog, contributing to the mitotic arrest of the male germline. On the other hand, PGD2 produced in Sertoli cells activates Notch1 and Cyp26B1 gene expression, indirectly influencing the germline fate.

In vivo analysis of double-knockout L/H-Pgds (L/H-Pgds<sup>−/−</sup>, i.e. depleted for PGD2) gonads showed that the proliferation rate of E13.5 mutant germ cells increased by 1.5-fold compared with WT germ cells. At E15.5 and even E17.5, nearly 10% of the mutant germ cells were still Ki-67 positive, showing that a significant proportion of the mutant germ cells were not mitotically arrested and were still engaged in the cell cycle at a time which should be quiescent (Moniot et al. 2014). Meanwhile, cell cycle inhibitors p21<sup>Cip1</sup> and p57<sup>Kip2</sup> are downregulated and cell cycle activators CyclinE1 and E2 are upregulated (Fig. 2), suggesting that PGD2 signaling is involved in the control of cell cycle genes in fetal testes, contributing to the arrest of mitotic process. Moreover, at late embryonic stages, the ectopic expression of pluripotency markers Pou5f1 (Oct4), Sox2, and Nanog was detected in L/H-Pgds<sup>−/−</sup> testes and the male germ cell marker Nanos2 is downregulated in mutant testes suggesting that PGD2 has a role in the germ cell differentiation in the embryonic testis. Somatic factors, Notch1 (Garcia et al. 2013) and Cyp26B1, an RA-metabolizing enzyme of the cytochrome P450 family that is produced by the Sertoli cells and that protects germ cells from RA (Bowles et al. 2006), were significantly reduced in E13.5 mutant gonads (Fig. 2), suggesting that PGD2 produced by...
Sertoli cells influences the differentiation of the embryonic germ cells (Moniot et al. 2014). Finally, the DP2 receptor is responsive to the effects of PGD2 in the male germline, because Dp2−/− testes have the same phenotype as that of the L/H-Pgds−/− testes (Fig. 2; Moniot et al. 2014). PGD2 signaling is thus an early pathway acting in both paracrine and autocrine manners (Fig. 2), contributing to the proper differentiation of male fetal germ cells.

Conclusions, perspectives

The development and maturation of the reproductive organs are complex and highly regulated biological mechanisms, in which numerous factors and signaling pathways are involved. In this review, we addressed the advancement of knowledge on PGD2 signaling in female and male reproduction, particularly in the formation of embryonic gonads and the maturation of adult reproductive organs. Whereas PGD2 signaling through both PGDSs is involved in the differentiation of the embryonic testis at the somatic and germ cell levels, its roles in steroidogenesis and spermatogenesis in adults are still under debate. The dual roles of the L-PGDS enzyme suggest that this protein plays a role in both the development and maturation of sperm and spermatogenesis. Seminal L-PGDS, an important carrier of bile pigments, retinoids, thyroid hormones, and essential fatty acids, would contribute to providing, beyond the blood–testis barrier, thyroid hormones, and retinoids to the developing germ cells in the seminiferous tubules and the maturing spermatozoa in the epididymis. Both PGDSs are indeed expressed in testes of patients with impaired spermatogenesis, suggesting their involvement in fertility (Leone et al. 2002).

The increasing incidence of disorders of the reproductive organs in men, such as cryptoorchidism, hypospadia, decreased semen quality, and testosterone concentration, or testicular cancers, has been observed in recent decades (Toppari et al. 1996, Skakkebaek et al. 2001). The use of NSAIDS drugs during the second trimester of pregnancy is associated with an increased risk of cryptorchidism in humans (Jensen et al. 2010) and in rats (Kristensen et al. 2011a). As PGD2 is a potential target for endocrine disruptors and NSAIDS, our findings thus open new perspectives for future investigations into how germ cell development can be perturbed by the external environment. Germ cells that are not controlled appropriately during fetal life can later transform into carcinoma in situ (CIS), the pluripotent precursor cells for testicular germ cell tumors (Kristensen et al. 2008). Indeed, PGD2/DP2 signaling is involved in the control of key regulators of the G1/S phase checkpoint and in the repression of pluripotent markers’ expression in the male embryonic germline; its ablation resulting in CIS-like phenotype in the mice gonad. Further work will determine whether the double L/H-Pgds mutation can lead to a high incidence of germ-line tumors in the 129sv background. PGD2 signaling through L-PGDS and SOX9 expression suppresses NT2/D1 cell migration and invasion, suggesting an important role for PGD2 in cancer cell suppression in the testis (Wu et al. 2012). L-PGDS is abnormally expressed in ovarian tumors (Su et al. 2003, Malki et al. 2007). The antiproliferative effect of PGD2 has been highlighted in human ovarian cancer cell lines (Kikuchi et al. 1986, Su et al. 2003); stimulation of the PGD2/DP1 signal transduction pathway upregulates SOX9 expression leading to the inhibition of cancer cells growth (Malki et al. 2007).

The recent findings, showing that endocrine disruptors and NSAIDS influence the PGD2 production in the testes and that PGD2 signaling is involved in multiple steps of the embryonic testis differentiation, might introduce this pathway in the etiology of the reproduction diseases. As the pharmacology of the PGD2 signaling is well documented, either activators of this pathway or DP1/DP2 agonists may be useful as new therapeutic agents.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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