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Chapter: Nuclear receptors in male fertility and related disorders.

New perspectives on PPAR, VDR and FXR α as new actors in testicular pathophysiology.

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Abstract

The incidence of reproductive disorders is constantly increasing and affects 15% of couples, with male's abnormalities diagnosed in almost half of the cases. The male gonads exert two major functions of the testis with the productions of gametes (exocrine function) and of sexual hormones (endocrine function). In the last decades, next to steroid receptors such as estrogen and androgen receptors, the involvement of other members of the nuclear receptor superfamily have been described such as Steroidogenic factor-1 (SF-1), Nerve growth factor IB (NGFIB), Liver-X-Receptora (LXR α and Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX-1). The purpose of this review is to highlight the emerging roles of some members of the nuclear receptor (VDR), Peroxisome Proliferator-activated Receptor (PPAR), Farnesol-X-receptor- α (FXR α). We discuss how these receptors could participate to explain male fertility disorders; and their potential to be use as biomarkers or therapeutic targets for management of fertility disorders.

People's health depends on integrated physiology which itself relies on the proper function of organs. The organ's integrity depends on cellular homeostasis; which results from the coordinated expressions of genes that are involved in vital cellular functions (metabolism, proliferation, cell death program). The expressions of these genes are modulated by multiple transcription factors. Among them, the superfamily of nuclear receptors (NR) has been defined to be involved in the regulation of physiological functions and has been associated with the appearance and the development of several pathologies. The NR superfamily represents one of the largest groups of transcription factors in eukaryotes.

In this review, we focus on the emerging roles of some members of the nuclear receptor superfamily on male reproductive function. This is of importance as the incidence of reproductive disorders is constantly increasing and affects 15% of couples. Among the NRs, the present review highlights data on the vitamin-D receptor (VDR), Peroxisome Proliferator-activated Receptor (PPAR) and Farnesol-X-receptor α (FXR α). We discuss how these receptors could be involved in male fertility disorders; and their potential to be use as biomarkers or as therapeutic targets for management of fertility disorders.

A/ The nuclear receptor superfamily

Nuclear receptors are transcription factors that are ligand activated. They regulate a variety of physiological processes by inducing the transcription of target genes. Among the receptors, the nuclear receptors appear in the last decades to play major roles in both physiology and pathologies. The NRs have been defined several decades ago with the identification of the steroid receptors. Extensive studied have been made by then and several members of the superfamily have been identified. These researches allowed identifying 48 members in humans. Since the beginning, scientists have made analyses in order to characterize the structure and the functions of the NRs.

A-1/ Structure of the NRs.

Almost all the NRs have the overall same structure. The NR's structure is divided into key functional areas (Giguère, 1999). The most variable part regarding size and protein sequence is the N-terminal part (also called domain A/B). It contains the activating function-1 (AF-1), which is responsible for the interactions of the NRs with co-activators and co-repressors. These interactions are independent of the binding of the ligand. However, this association with the co-factors is involved in the optimal transcriptional activity of the NRs.

The second domain corresponds to the internal central domain. It is the highly conserved in the group. This domain contains two zinc fingers, which are involved in part to the DNA binding. This is an essential element as NRs act as transcription factors *via* the binding to specific promoter sequences called hormone response elements (HRE). Depending whether the NRs act as monomer or dimers, these motifs are half-sites of six bases or two half-sites separated by 0 to 5 bases. The HRE are organized in direct (DR, direct), inverted (IR, inverted), or mirrored (ER, everted) repeats.

A hinge region connects the DNA binding domain (DBD) to the ligand-binding domain (LBD). This causes the rotation of the LBD to allow the binding of the dimer on the HREs. In addition, this region contains the nuclear localization signal (NLS), which transfers the receptor to the nucleus.

Finally the last domain corresponds to LBD. This confers the specificity and selectivity of the ligand response. This C-terminal domain has second transcription activation domain (AF-2). The AF-2 acts in a ligand-dependent manner for the attachment of co-activators.

NRs activate or repress gene transcription. Transcriptional activation is dependent of the ligand binding. NRs recruit co-activator proteins leading to the recruitment of a larger complex with histone modifying enzymes such as histone acetyltransferases or histone methyltransferases and then of transcriptional machinery.

The inhibition of transcription by the NRs relies on the interaction with co-repressors that recruit histone deacetylases (HDACs).

A-2/ Classification of the NR superfamily.

Among the different classifications of nuclear receptors that have been proposed, here we highlight the one relying on functional point of view. In that case, there are three main categories of nuclear receptors: the endocrine receptors, the orphan nuclear receptors "adopted" with low-affinity ligand, and the nuclear orphan receptors.

<u>* The endocrine receptors</u>. These receptors respond to steroid hormones as ligands, and they have a high affinity for their ligands. Among these NRs, there are the androgen, estrogens, progestin, glucocorticoid, and mineralocorticoid receptors. These NRs are associated with heat-shock proteins within the cytoplasm. The arrival and the binding of the ligand lead to the departure of heat-shock proteins. Then, the NR could act though cytoplasmic signaling pathways in interaction with MAPK, PI3K or ERK pathways. In another way, the NRs can translocate to the nucleus and dimerize. The endocrine receptors bind as homodimer to HRE, consisting of two inverted repeats, in the 5'-regulatory sequences of their target genes. They regulate gene

expressions through the recruitment of transcriptional co-activators.

<u>* The adopted receptors.</u> It has been suggested several years ago that these NRs are permanently located on their response elements. This point could be now amended as some of these NRs could have cytoplasmic activity. When bind to the DNA, in absence of ligand, NRs are associated transcriptional co-repressors in order to repress the transcription. The arrival of the ligand leads to modulation of the NR conformation, which is associated with the release of co-repressors and recruitment of co-activators. This class of nuclear receptors includes members, such as LXR α , β (liver X receptor α , β) and peroxisome proliferator-activated receptor (PPAR), which act as heterodimer with the relinoid X receptor (RXR). Others, such as VDR, act as homodimers. However, there is evidence that most of these NRs are also capable of acting as homodimers or monomers.

<u>* The orphan receptors.</u> This corresponds to NRs for which no natural ligands have been identified such as estrogen-related receptors (ERR, NR3B), steroidogenic factor 1 (SF1; Nr5A1).

This category also contains DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1; NR0B1) and SHP (Small Heterodimer Partner; NR0B2), which are quite closed. Both NRs lacks the classical DNA-binding domain (DBD) (Seol et al., 1996). They act as a negative regulators of transcription *via* the interactions to other nuclear receptors and the recruitment of co-repressors or histone methyl transferases (Båvner et al., 2005).

B. The testicular physiology.

The testis, the male gonad, is composed of different types of cells in order to ensure the synthesis of hormones (endocrine function) and the production of spermatozoa (exocrine function). As the NRs highlighted in this review are expressed in different cell types of the testis and could thus participate to control testicular functions, we report here a short overview of testis physiology.

B-1/ Testicular physiology.

The Leydig cells ensure part of the endocrine function of the testis with the synthesis of the male sexual hormone, namely testosterone. The testes also produce estrogen and progesterone. Testes produce the major quantity of androgens in mammals. Steroid hormones are produced from cholesterol during steroidogenesis *via* a multi-enzymatic process (Hu et al., 2004, p. 20)(Midzak and Papadopoulos, 2015). The testicular endocrine function is under the control if the hypothalamo-pituitary axis (Shacham et

al., 2001). It is well-known that sex steroids is exert a negative feedback on the hypothalamopituitary axis to avoid over production of steroids.

Steroids are critical for many physiological functions related to urogenital tract or development of muscle or bone for examples. During the embryonic development, the fetal Leydig cells secrete a first peak of testosterone that is associated the urogenital tract masculinization. Then later on at puberty, the increase of testosterone production is responsible of the establishment of secondary sexual characteristics and the beginning of spermatogenesis. This is in part supported by maturation of Sertoli cells in response to androgen signaling. Finally, at adulthood, testosterone concentrations are critical to ensure the efficiency of spermatogenesis (survival and differentiation of germ cell lineage) and the maintenance of the normal genital tract (prostate, epididymis, seminal vesicle). The critical functions of steroids like androgens have been highlighted by human diseases such as androgen resistance due mutations within the androgen receptor gene (AR; NR3C4) (Oakes et al., 2008) and the analysis of rodent transgenic models. Next to the androgens, estrogens act through the nuclear receptors Er-alpha (ER α ; Esr1; NR3a1), ER-beta (ER_β; Esr2, NR3A2) and via the G-protein-coupled receptor (GPCR; GPR30) (Carreau et al., 2011) (Hess and Cooke, 2018). In that line, over the last decades, many reports in both human and rodent models have highlighted that exposure to exogenous molecules, so called endocrine disrupters, are associated with testicular abnormalities and male fertility disorders (Palanza et al., 2016; Sedes et al., 2018).

The exocrine function of the testis relies on the interactions between almost all the cell types of the male gonad. It is ensured within the seminiferous tubules, where Sertoli cells and germ cells cooperate to achieve the spermatogenesis.

Sertoli cells are key component of the seminiferous tubules. They are involved in the nutritional and structural support of the germ cell lineage to maintain the integrity of the epithelium. One main function of Sertoli cells is to generate the blood-testicularbarrier (BTB). It is established at puberty following the response to diverse signaling pathways such as androgens, retinoids and thyroid hormones (Tarulli et al., 2012). The structural function of the BTB is ensured by tight junctions (occludin, zonula occludens), adherens junctions (cadherins, catenins, nectines, laminins, integrins) and gap junctions (connexin) (Cheng and Mruk, 2012). The BTB is also involved in the nutritional function of Sertoli, as sugars, amino acids, hormones and electrolytes could be transferred to neighboring cells. The BTB also confer an immune context to protect post-meiotic cells that have been generated after the establishment of the immune system. The dynamic of cell-cell interactions between Sertoli and germ cells are critical for germ cells progress within epithelium and then their release at spermiation. This process relies on the synthesis of proteases and protease inhibitors by Sertoli cells (Lui et al., 2003).

The Sertoli cells support the complex coordinated process of spermatogenesis that gives spermatozoa. The length of a spermatogenesis wave is different between species with 35 days in the mouse and 74 days in humans.

The activity of spermatogenesis throughout life relies on the well-established balance between self-renewal of spermatogonial stem cells and differentiation. Under the control of multiple stimuli, germ cells enter a differentiation path. Spermatogonia undergo mitosis to produce primary spermatocytes (Spermatocyte I). Then meiosis is initiated at puberty giving rise to round spermatids, which then undergo new biochemical and morphological reshuffles giving rise to spermatozoa. Among these changes, the replacement of most histones by protamines (Cho et al., 2001) allow the compaction of chromatin (Dadoune, 2003) that is essential for the germ cell DNA protection and for the establishment of sperm head morphology. In addition, the flagellum and axonema are set up; both structures are essential for the movement of spermatozoa. Final maturation of the spermatozoa occurs during the epididymal transit and in the female genital tract (Bedford, 1970).

The incidence of reproductive disorders is constantly increasing and affects 15% of couples. Male's abnormalities are diagnosed in half of the cases. The increased incidence of pathologies of the male urogenital tract has been associated with exposures to environmental molecules. In addition, lifestyle is also involved in fertility issues such as smoking or diet. In that line, metabolic syndrome (MetS) has been linked with several abnormalities including overweight, dyslipidemia, hypertension and impaired glucose metabolism. The complexity of the interactions between metabolism and male fertility has been described in the last decades. Among all the consequences of MetS, male reproductive function is altered in some obese and diabetic patients. The relation between MetS and male reproductive function is still being studied to be fully deciphered. It has appeared that metabolism plays a major role in the regulation of testicular physiology and associated pathologies. In that line, male reproductive capacities depend on testicular glucose homeostasis. Altered glucose levels cause adverse effects on male fertility *via* the alterations of testicular cells types, sperm motility, and ingredients of seminal

plasma. Diabetes is associated with the increase of oxidative stress that lead to causing sperm DNA damages (Shi et al., 2017).

In all these case, regardless the origin of testicular disorders, the alterations of testicular physiology can participate to multiple pathologies such as alterations of male sexual maturation during puberty and fertility disorders. In addition, it has been demonstrated that alterations of germ cell quality could be associated with transmission of abnormalities to progenies even over several generations. These transmissions are supposed to be driven by altered epigenetic patterns of sperm cells. It could be associated with multigenerational programming of pathologies such as fertility disorders or metabolic diseases (Aiken et al., 2016; Anway et al., 2005; Baptissart et al., 2018).

Next to fertility disorders, alterations of germ cells could be associated with testicular germ cell cancers (TGCT). TGCT is a rare solid tumor that accounts for 1% of all cancers in men. Testicular germ cell tumors are the most frequently diagnosed cancer in young men (15-39 years old) (Batool et al., 2019; Rajpert-De Meyts, 2019). The development of TGCT might be influenced by the exposures to chemicals with endocrine disrupting properties. Of the major influences described, ethnicity and familial history of TGCT are known to be associated with increased TGCT risk. It is estimated that 40% of TGCT are of genetic origin (Litchfield et al., 2015).

B.2/ Peroxisome proliferator-activated receptors & testis.

The Peroxisome proliferator-activated receptors (PPAR) are members of the nuclear receptor superfamily and have been demonstrated to regulate lipid homeostasis and tumorigenesis for examples. There are three isotypes of PPARs named PPAR α , PPAR β/δ and PPAR γ . Within the testis, all PPARs are expressed in both somatic and germ cells (Froment et al., 2006). PPAR β/δ are expressed in Leydig, Sertoli and germ cells. In the testis, PPAR γ protein is detected at high level in Sertoli cells and a weak expression is noticed in spermatocytes. Ppar γ mRNA was highly expressed in late spermatids, while Ppar β mRNA was highly expressed in round spermatids. Consistently, FACS cell sorting experiments have shown that PPAR γ mRNA is found in M2A positive germ cells within first trimester human fetal testes (Muczynski et al., 2012).

The most defined expression pattern is the one of PPAR α . In the developing rat testis, PPAR α is detected since the first days after birth in tubular and interstitial cells. In addition, PPAR α is expressed in interstitial Leydig cells and in tubular cells mainly in meiotic spermatocytes, spermatids as well as in the peritubular cells within adult human testis.

These patterns of expression suggest that PPARs might play some role in testicular physiology. This is sustained by the phenotypes observed in mice deficient for these receptors or in experiments using stimulation of wild-type males by specific modulators. PPAR α - and PPAR β / δ -null mice are viable and fertile. However, some data have suggested their roles in gamete maturation.

PPARs in Leydig cells.

PPARγ acts as an important molecule for regulating energy homeostasis. It modulates the hypothalamic-pituitary-gonadal (HPG) axis, and is reciprocally regulated by the HPG.

Inflammation alters testicular steroidogenesis and spermatogenesis, and in turn male infertility. It has been suggested that PPAR transcriptional activity disturbs the expression of both estrogen and androgen receptors and impair testicular steroidogenesis and ROS metabolism in the context of LPS-induced inflammation (Wang et al., 2020).

Using a combination of pharmacological approaches, it has been demonstrated that in Leydig cells, PPAR α and PPAR γ , in interaction with the signaling pathway of the G-protein coupled estrogen receptor (GPER), regulate steroidogenesis through the control of lipid homeostasis. These collaborative pathways also regulate morphology, migration competences and cytoskeleton structure of Leydig cells (Gorowska-Wojtowicz et al., 2018).

Over the last decades, it has been widely defined that endocrine disrupters, through the alterations of hormonal homeostasis, impact testicular physiology and male fertility. Some of these endocrine disrupters have been identified as PPAR ligands. In that line, it has been demonstrated that 2,4-dichlorophenoxyacetic acid (2,4-D), a commonly used herbicide and a possible endocrine disruptor is a modulator of peroxisome proliferator-activated receptor- α . Recent data have demonstrated that the deleterious impacts of 2,4-D are in part mediated by PPAR α . Indeed, exposure to 2,4-D leads to a decrease of testicular testosterone concentration, which in turn results in Sertoli and germ cells alterations and fertility disorders. Consistent with the data from Gorowska-Wojtowicz, PPAR α mainly acts through the downregulation of the expressions of genes involved in lipid homeostasis. These effects of the 2,4-D are mediated by PPAR α as no effects are observed in *Ppar\alpha*-null males (Harada et al., 2016).

Regarding fetal testis developmental, interaction of xenobiotics such as diisobutyl phthalate (DiBP) with PPARs leads to reduced anogenital distance, testosterone production and testicular expression of Insl-3 and of genes related to steroidogenesis. Moreover, PPAR α expression is lowered by Diisobutyl phthalate (DiBP) (Boberg et al., 2008).

Interestingly, it has also been proposed that PPARs expressions are altered in samples of Leydig cell tumors. In human, there is a downregulation of PPAR in LCT (Kotula-Balak et al., 2020a, 2020b).

PPAR and Sertoli cells.

Within Sertoli cells, multiple signaling pathways control PPAR expressions. For example, an up-regulation of PPAR α mRNA could be observed after stimulation with Follicle-Stimulating Hormone (FSH), and PPAR γ is up-regulated after lipopolysaccharide (LPS)-treatment. Moreover, some data demonstrate that exposures to xenobiotic ligands of PPAR γ and PPAR α induce their translocation to the nucleus.

Sertoli cells play a central role in spermatogenesis. They maintain the blood-testis barrier, an essential feature of seminiferous tubules, which creates the proper environment for the occurrence of the spermatogenesis. However, this confinement renders germ cells almost exclusively dependent on Sertoli cells nursing function and support. In that line, the control of lipid metabolism and energetic metabolism in Sertoli cells plays a critical role for germ cell homeostasis. Sertoli cells oxidize fatty acids to fulfill their own energy requirements. Sertoli cells phagocyte and degrade apoptotic germ cells resulting in increasing lipid content. Fatty acids are stored as triacylglycerides within lipid droplets. Thus Sertoli cells provide nutritional support for germ cell development as they actively metabolize glucose and convert it to lactate.

One of the main functions of PPARs in Sertoli cells is to control fatty acid metabolism. Pharmacological approaches have shown that of PPAR α and PPAR β/δ regulate fatty acids (FA) metabolism through the expression of CD36, carnitine palmitoyltransferase 1, long- and medium-chain 3-hydroxyacyl-CoA dehydrogenases. In the same line of evidence, it has been demonstrated that PPAR γ increased triacylglycerides levels and lipid droplet content accompanied by a rise in the expression of genes for proteins involved in fatty acid storage, such as the fatty acid transporter Cd36, glycerol-3-phosphate-acyltransferases 1 and 3, diacylglycerol acyltransferase 1 and perilipins 1, 2 and 3, all proteins that participate in lipid droplet formation and stabilization. In addition, data have defined that PPAR γ significantly elevated the expression of carnitine palmitoyltransferase (CPT)-1A, a key enzyme involved in fatty acid oxidation. The results indicated that PPAR γ reduced palmitoyl-induced lipid accumulation by promoting fatty acid oxidation mediated by CPT1A. PPAR γ activation leads to cytoprotection of Sertoli cells caused by dyslipidemia.

In addition, PPAR β/δ activation increases lactate energetic metabolism of Sertoli cells and germ cells. In addition, PPAR γ activation increases lactate production, accompanied by an increase in glucose uptake and Glut2 expression. These results suggest that PPAR γ activation in Sertoli cells participates to the regulation of lipid storage and lactate production thereby ensuring simultaneously the energetic metabolism for the Sertoli and germ cells.

One of the main roles of PPPAR δ is sustained by the fact that Ppar δ deficient mice (Ppar δ -/-) show smaller litter size compared to wild-type littermates. PPAR δ controls several genes such as claudin-11, p27, cyclin D1, and cyclin D2 leading to alterations of Sertoli cells proliferation, maturation and formation of tight junctions. It is thus suggested that Ppar δ regulates spermatogenesis in part through the modulation of Sertoli cell functions.

In the context of exposures to endocrine disrupters, such as phthalates, lead to testicular degeneration. It has been suggested that part of the effects of phtalates could be mediated by PPARs within the Sertoli cells

PPARs and Germ cells.

In human, PPAR γ protein is highly expressed in ejaculated spermatozoa, implying a possible role of PPAR γ signaling in regulating sperm energy dissipation. Its activation can be induced during capacitation and the acrosome reaction. *PPAR\gamma*, increases the viability of spermatozoa (Liu et al., 2015). In addition, PPAR α regulates the beta-oxidation of lipids. This is critical for the control of fatty acid and phospholipid metabolisms in germ cells. This is essential as lipid composition is key to modulate for sperm cell mobility and its viability (Douard et al., 2003).

PPARs & Endocrine disrupters

Many xenobiotics molecules such as herbicides, plasticizers, or components of personal care products have been defined as potential activators of PPARs. This abnormal activation of the receptors must leads to altered testicular functions that are driven by PPARs. However, not that much is known regarding the impacts of the exogenous activation of PPARs on reproduction. Environmental and dietary exposure to bisphenol-A (BPA) and its toxicological consequences are extensively reported. BPA has multiple cellular targets. Thus it suggests that the role of PPARs in BPA-induced effects on male reproductive functions must be better defined.

PPARs and Therapeutics

The identifications of the involvements of PPAR signaling pathways in pathologies led to the development of specific drugs to target these receptors in order to cure several diseases such as cardiovascular function, neurodegeneration, inflammation, cancer, fertility, and reproduction, and it is well established for managing dyslipidemia, diabetes, insulin resistance, and metabolic syndrome. Many *PPAR* synthetic ligands are used in current clinical drug applications. However, the effects of natural and synthetic PPAR ligands need to be better defined regarding reproductive toxicology and assisted reproductive technology.

Not that much is known about PPARs in testicular germ cell cancer. Dogs are models of spontaneous testicular cancers and it has been demonstrated that PPAR- α , - β and - γ levels are higher in TGCT than in normal testicular tissue. In human, PPAR γ is also more strongly expressed in human testicular cancer cells than in normal testicular tissues. Moreover, the use of PPAR γ ligands has anti-proliferative effects on testicular cancer cells (Hase et al., 2002).

B.3/ Vitamin-D receptor and Testis.

Vitamin D is a key regulator of calcium and phosphate homeostasis and bone physiology. In addition, other targets have been identified such as reproductive organs. Vitamin-D deficiency impairs testicular development and spermatogenesis in mice (Cito et al., 2020; Fu et al., 2017).

Vitamin D is synthetized in the skin as a result of UV-B radiation. It then binds to its nuclear receptor VDR, which has been identified many years ago. More recently, it has been established that vitamin D initiates rapid cellular responses through a putative plasma membrane-associated receptor (VDRmem).

The metabolism of Vitamin-D relies also on the expression of the vitamin D metabolizing enzymes such as the cytochrome CYP2R1, CYP27B1, and CYP24A1. The expression of the vitamin-D metabolizing enzymes is important as their presence and activity regulate the intracellular concentration of 1α ,25(OH)₂D₃ and then VDR activation. These enzymes and VDR are expressed in the organs of the male reproductive system. In adult testis, they are mainly expressed in the germ cells. This suggests the potential roles of Vitamin-D on spermatogenesis. The expression of VDR has been observed in immature Sertoli cells including fetal Sertoli cells. In addition VDR and the vitamin-D metabolizing enzymes are also expressed in the hormone producing Leydig cells, which suggests a direct link between vitamin D and sex steroid production.

The role of Vitamin-D signaling pathways on male fertility has been highlighted by the study of vitamin-D deficient rats as well as the use of rodent models of VDR null males. Indeed, these models show altered spermatogenesis and decreased sperm count and motility. It has also been demonstrated that the decrease of Vit-D during aging might be associated with testicular aging, which leads to a decrease in spermatogenesis and steroidogenesis. Vitamin-D signaling pathways have been identified to regulate key functions in different testicular cell types such as Leydig, Sertoli and germ cells.

VDR & Leydig cells. .

Vitamin-D plays an important role in reproduction by modulating testicular steroidogenesis. Vitamin-D treatment of aged rats resulted in significant improvement in sperm parameters, testicular histology, serum testosterone, and estrogen levels (Jeremy et al., 2019a, 2019b). In aged rat models, it has been defined; using *in vivo* and *in vitro* experiments, that vitamin-D directly regulates testicular steroidogenic function. Vitamin-D controls the expression of the aromatase encoding gene *Cyp19a1* as well as the expression of the androgen receptor gene. These data highlight that Vit-D signaling pathways control the activities of sex steroids, which ensure spermatogenesis during aging. Consistent with these data, Vitamin-D seems to impact steroid production in human (Holt et al., 2020).

VDR & Sertoli cells.

Vit-D homeostasis and signaling have been demonstrated to play some roles in Sertoli cells. Vit-D acts through its conventional nuclear receptor and through non-genomic effects (Menegaz et al., 2011). It has been suggested that Vit-D signaling pathways might regulate Sertoli cell secretory activities, and in turn play some role in the control of spermatogenesis. However, further studies will be necessary to decipher the role of VDR receptors in Sertoli cells through the use of specific invalidation of the *Vdr* gene using the recombinase Cre under the control of the AMH sequences.

VDR and Germ cells

It has been demonstrated that in an aging rat model, Vit-D increases cell proliferation and decreases apoptosis. This is associated with a lower level of oxidative stress and modulation of the advanced Glycosylation End-Product Specific Receptor (AGER). Thus during aging, the altered lower levels of Vit-D impacts testicular physiology through the control of balance between the proliferation and apoptosis processes (Jeremy et al., 2019a, 2019b).

Interestingly, it has been recently demonstrated that vitamin-D induces modulation of DNA methylation within sperm cells (J et al., 2018), which could thus be hypothesized to impact male fertility.

This impact of Vit-D/VDR signaling pathways in germ cell has also been demonstrated in

human as both VDR and Vit-D-metabolizing enzymes are expressed in human testis and spermatozoa. In addition, the expression of the cytochrome CYP24A1, an enzyme involved in the inactivation of Vit-D, has been reported in human spermatozoa. Moreover, the level of CYP24A1 has been suggested to be a marker to distinguish normal and infertile men. It has been defined that Vit-D leads to an increase of Calcium (Ca2+), which seems to be associated with the improvement of sperm motility and of the acrosome reaction *in vitro*. In that line, some epidemiological studies highlight the positive association between serum 25-hydroxy-vitamin D (25(OH)Vit-D) concentrations and sperm motility in both fertile and infertile men. However, there are still some divergences regarding the association between Vit-D levels and reproduction parameters such as androgen concentrations, as some studies report linear correlation and other suggest a potential inverse U-shaped association. Indeed, men with both low and high 25(OH)Vit-D concentrations demonstrate poorer gonadal function compared with those with intermediate concentrations. This is essential to better define the type of association regarding putative treatment of patients with Vit-D supplementation (Karras et al., 2016).

VDR & germ cell cancer.

Testicular germ cell tumours are the most common malignancy in young men aged 18-35 years. They are clinically and histologically subdivided into seminomas and non-seminomas. Altered levels of VDR expression have been reported over the last years in several cancers. In the same line, VDR is found at both mRNA and protein levels in testicular germ cell cancers. In addition, it has been established, using cell lines of testicular germ-cell cancers, that treatment with Vit-D inhibits proliferation of TGCT cell-lines. These data have open research perspectives to define whether targeting VDR signaling pathways could be used in the treatment of germ cell cancers (Bremmer et al., 2012).

B.4/ Farnesoid-X-receptor and testis.

Bile acids and their signaling pathways have emerged in the last decade as regulators of male reproductive function. Bile acids act through the modulation of specific receptors. BA acts mainly through FXR-alpha (Farnesoid-X-Receptor-alpha; NR1H4) and the G-protein coupled membrane receptor (GPBAR-1; TGR5). In the context of this review article on nuclear receptors, this part focuses on the roles of FXRα within testicular physiology.

FXR α is expressed in testis from *in utero* to adult life in both mouse and human. The use of mouse models helped to decipher that in neonatal life, FXR α is expressed in germ cell (Martinot et al., 2017a) and that in the adult testis, it is expressed in the interstitial space and

mainly in Leydig cells (Volle et al., 2007). Combined these data suggest that FXR α participate to the control of the endocrine and exocrine functions of the testis.

$FXR\alpha$ and Leydig

Few years ago, androsterone, a metabolite of testosterone, was characterized as n agonistic modulator of FXR α reinforcing the idea that FXR α could control testicular androgen synthesis (Wang et al., 2006).

In adult mice, FXR α activation by a synthetic agonist (GW4064) results in a decrease of androgen production. This leads to a lower expression of steroidogenic genes as *Star*, *Cyp11a1* or *3β-Hsd* (Volle et al., 2007). Within the Leydig cells, FXR α induces the transcription of the genes encoding the small heterodimer partner (SHP; NR0B2); which then inhibits the expression and the activity of LRH-1and SF-1, two known regulators of the steroidogenesis. Such negative effects of FXR α signaling on steroidogenesis was reported in boar taint (Gray and Squires, 2013).

Interestingly, it has been demonstrated that in experimental models where bile acid levels are dramatically increased such as bile-duct ligation lower testosterone levels are observed as well as altered puberty phenotypes (Van Thiel et al., 1985). The roles of bile acid signaling on puberty onset and male sexual maturation were defined (Baptissart et al., 2016). Pubertal exposures to bile acids increase the percentage of sterile males at adulthood. This decrease of fertility was correlated with a lower production of spermatozoa; which results from a loss of germ cell via apoptosis. This later is a consequence of a decreased testosterone synthesis. Indeed, it was observed that testosterone supplementation restores spermatogenesis. The use of *Fxra* deficient mice validate the main role of FXRa in these processes. At the molecular level, it has been demonstrated that during puberty, FXRa controls the expression of Dax-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1; NR0B1), a known repressor of steroidogenesis (Baptissart et al., 2016). These *in vivo* data clearly demonstrate the impacts of FXRa on androgen steroids. In addition it has been shown that, at least *in vitro*, expression of the aromatase gene as well as estrogen levels are both controlled by FXRa (Catalano et al., 2010).

These data suggest that FXR α exerts a local control of the testicular steroidogenesis. Recently, FXR α activation was shown to repress the expression of the LH/CG receptor, namely *Lhcgr* (Baptissart et al., 2016). The FXR α signaling pathway alters LH/CG sensitivity of Leydig cells both *in vitro* and *in vivo* suggesting that FXR α might interferes with the hypothalamopituitary axis (HPA), which is the main regulator of testicular physiology. This crosstalk between HPA and FXR α must be quite complex as LH/CG, through protein kinase-A signaling, increases the expression of the Fxr α (Holota et al., 2019).

All the above data suggest a complex negative feedback of steroid synthesis involving the hypothalamo-pituitary-gonadal axis and FXR α . These are important findings as the control of the Leydig cell functions is predominantly mediated by the hypothalamo-pituitary-gonadal axis *via* LH/CG. It highlights that FXR α might act as an important actor of the regulation of testicular physiology during puberty. This is of interest in order to identify the etiology of primary hypogonadism.

In addition, within the Leydig cells, it has been demonstrated using R2C Leydig tumor cell line that FXR α signaling controls the proliferation and the apoptotic processes of Leydig cells. At the molecular level, FXR α regulates the expression of the proliferation marker Ki-67 and the expression of Tp53 (Catalano et al., 2013).

$FXR\alpha$ and Sertoli.

So far, FXR α has not been detected within the Sertoli cells. However, recent data show that Sertoli cells functions are altered in Fxr $\alpha^{-/-}$ males (Martinot et al., 2017b). Indeed, during early neonatal life in mice, the number of Sertoli cells was higher in Fxr $\alpha^{-/-}$ males in wild-type controls. This was also observed in older mice (up to 12 months). However, no difference was observed in the establishment of a functional blood-testis barrier (BTB).

$FXR\alpha$ and Germ cells.

 $FXR\alpha$ has been demonstrated to be expressed in spermatogonia (Martinot et al., 2017b) where it seems to play critical role in the establishment and the maintenance of spermatogonial stem cell pool. This ensures reproductive capacity.

Recently, using $Fxr\alpha^{-/-}$ mice, unexpected involvement of $FXR\alpha$ on germ cell physiology independent of its effects on somatic cells have been defined. The data reveal that $Fxr\alpha^{-/-}$ males maintain reproductive capacities during aging compared to wild-type control mice. This was associated with a greater production of spermatozoa by the $Fxr\alpha^{-/-}$ mice. This maintenance of fertility capacities results from a higher number of undifferentiated germ cells during aging that ensures a better efficiency of spermatogenesis. It was then decipher that $FXR\alpha$ is involved in the establishment and maintenance an undifferentiated germ cell pool during the neonatal life, and in turn influences male fertility. $FXR\alpha$ regulates the expression of several pluripotency factors. Among these, *in vitro* approaches show that $FXR\alpha$ controls the expression of the pluripotency

marker Lin28 in the germ cells.

The $Fxra^{-/-}$ males show an early entry into meiosis compared to wild-type males. It might be correlated to the modulation of the retinoid metabolism suggested by the lower expression of retinoid-degrading enzyme *Cyp26b1*.

It has also been demonstrated that FXR α is expressed in human sperm cells (Malivindi et al., 2018). Immunofluorescence approaches show that FXR is located in the middle piece of spermatozoa. Data showed that increasing concentrations of CDCA negatively impact sperm parameters. The use of FXR α agonist is associated with increased triglyceride levels. This leads to an alteration of metabolic reprogramming of the capacitated sperm. In human sperm cells, FXR α signaling has a negative impact of fertilizing activity. It might be hypothesized that bile acid levels could be involved in infertility related to cholestatic pathologies either in males or females. Combined data in mouse and human showed that the lack or inhibition of FXR α in germ cells, either spermatogonia or sperm cells must be a way to improve germ cell homeostasis. Thus FXR α status and the bile acid levels could be good diagnosis or prognosis markers of fertility and it could be propose to use FXR α as a therapeutical target through the use of antagonist or blocking antibody to improve germ cell capacities at least in cases of pathologies where bile acid levels are increased.

It has been demonstrated that some testicular cells produced primary bile acids (Martinot et al., 2017a). This local testicular synthesis of BA is altered in Fxrα-/- males. This alteration is even worth Fxrα-/- males fed a diet supplemented with BA. This was associated with a drastic germ cell loss in few weeks. It has been demonstrated that in these mice there is an accumulation of intermediate metabolites of BA synthesis, which were previously described as CAR and PXR modulators. Such BA metabolites can also been found in human (Martinot et al., 2017a). Recent data have shown the critical role of the Constitutive-Androstane-Receptor in this phenotype (Martinot et al., 2017a). These data open interesting perspectives to better define how BA homeostasis contributes to physiological or pathophysiological conditions *via* the modulation of CAR activity.

$FXR\alpha$ and endocrine disruptors.

All the above data highlight the important roles of FXR α in the regulation of the testicular physiology and its associations with testicular abnormalities. Interestingly, over the last decades, environmental molecules such as endocrine disrupters (ED) have been suspected to be a cause of fertility disorders and of testicular dysgenesis syndrome. EDs with estrogenic activities seem to be deleterious for testicular physiology. Interestingly, FXR α interacts with estrogenic signaling

pathways (Baptissart et al., 2013). Recently a paper from Sèdes *et al.* showed that modulation by Bisphenol-A, an estrogenic ED, and by stigmasterol, an antagonist of FXR α lead to impaired fertility through a lower sperm cell production, which is associated with the alteration of the establishment and maintenance of the undifferentiated germ cell pool (Sedes et al., 2018). This interaction between both pathways seems to result from the downregulation of *Fxra* expression following BPA exposure. Interestingly, some effects of the combination of BPA and Stigmasterol exposures were also observed in human testis (Sedes et al., 2018). These data show that FXR α activity could be a mediator of the effects of ED on testicular physiology even in human.

C. Conclusion & theraputical perspectives.

Infertility is a major public health issue defined by the World Health Organization as the inability of a couple to conceive after 1 year of unprotected regular intercourse. Today, around 15% of couples around the world are affected by infertility (Agarwal et al., 2015); the male factor being totally or partially involved in 30 to 50% of cases.

Male infertility can be divided into 2 categories: abnormalities of excretory origin, which define a defect in the delivery of sperm in the genital tract, and abnormalities of secretory origin, which describe an alteration in the production of gametes by the male gonads.

Male fertility disorders and testicular damages have been correlated with adverse conditions such as exposures to environmental molecules (EM, including endocrine disrupters) or metabolic disorders (Bellastella et al., 2019; Foresta et al., 2018; Leisegang and Dutta, 2020). However, 30% of male fertility disorders remain unexplained (García-Baquero et al., 2020). It is therefore necessary to better understand the physiology of the testicle in order to identify the causes of infertility that are still idiopathic.

Testicular physiology is modulated by numbers of molecules through the interactions between the different cell types of this organ through as paracrine, autocrine or endocrine factors. Alterations in the homeostasis of theses cell types and their secreted factors could lead to the development of pathologies. As the nuclear receptors bind small molecules capable of regulating their biological activity, they are targeted by numbers of physiological molecules and also by exogenous molecules such as endocrine disrupters. In that line, numerous studies have highlighted the critical roles of some nuclear receptors in testicular physiology and pathologies. Advances in the identification of physiological ligands of nuclear receptors have helped the scientific community to decipher their roles in physiology and diseases. The knowledge of their mechanisms of action is essential as several NRs activation and inhibition could have opposite or identical effects on particular molecular mechanisms (gene regulation). One of the main

objectives in the field of nuclear receptors is thus to gain a better understanding of the mechanisms underlying their actions in order to selectively modulate their activities in specific tissues or cell types. In addition, the characterization of the impacts of nuclear receptors during the utero/neonatal period is a challenge, because such exposures could lead to immediate diseases, but also to diseases later on in adulthood. The identification of the precise involvements of the NR signaling pathways could help to discover new diagnostic and/or prognostic tools concerning the etiology and/or progression of the diseases. In that line, polymorphisms of these nuclear receptors could be researched to define diagnosis/prognosis marker regarding testicular pathophysiology related at least for fertility disorders.

The data review here sustained the main emerging roles of PPAR, VDR and FXR in the male reproductive functions and particularly through the control of testicular physiology. These receptors are thus important actors to take into consideration to enlarge our knowledge of male fertility. These major involvements have been defined using experimental models, pharmalogical approaches and also studies on human biological samples related to diseases.

In addition, nuclear receptors could be considered in the future as targets, as could be suggested for PPAR or FXR, to be modulated during assisted procreation such as *in vitro* fecundation to increase the efficiency of the protocols

Since the activity of nuclear receptors can be modulated by ligands, they are important putative targets for drug development, prompting pharmaceutical companies to make efforts in this regard. NR could be important for offering personalized medicine in the context of specific diseases such as fertility disorders.

In the last decades, at least PPAR and FXR α appear as key mediators of the impacts of environmental molecules, such as endocrine disrupters. These data have opened up new research perspectives in a fascinating research theme, which poses major problems for present and future generations.

Even if there is still a long way to go, the different aspects of the impacts of NR on physiology and/or pathologies highlighted by this review support the idea that this area of research will continue to fascinate researchers with impacts on clinical approaches to improve human health.

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