



# Cholesterol: A Gatekeeper of Male Fertility?

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Cholesterol is essential for mammalian cell functions and integrity. It is an important structural component maintaining the permeability and fluidity of the cell membrane. The balance between synthesis and catabolism of cholesterol should be tightly regulated to ensure normal cellular processes. Male reproductive function has been demonstrated to be dependent on cholesterol homeostasis. Here we review data highlighting the impacts of cholesterol homeostasis on male fertility and the molecular mechanisms implicated through the signaling pathways of some nuclear receptors.

**Keywords:** cholesterol, fertility, testis, spermatozoa, nuclear receptors

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Infertility is a major public health issue defined by the World Health Organization as the inability of a couple to conceive a child after 1 year of unprotected regular sex. About 15% of couples worldwide are reported to be infertile (1); and male disorders are diagnosed in 30–50% of cases (2). Azoospermia is one of the most severe case of infertility; which is defined by the total absence of spermatozoa in the ejaculate and affects more than 20% of infertile men (3).

Male infertility can be divided into two categories: abnormalities of excretory origin, which define a defect of sperm delivery in the genital tract and abnormalities of secretory origin, which correspond an alteration of the production of the spermatozoa by the testis. The aetiologies of testicular lesions involve multiple genetic, environmental and/or behavioral factors. However, in 30% of cases, male fertility disorders remain unexplained (4). It is therefore necessary to better understand the physiology of the testis in order to identify the causes of infertility that are still idiopathic.

Male reproductive function has been demonstrated to be highly dependent on cholesterol homeostasis. Indeed, cholesterol is the precursor of steroid synthesis which is crucial for normal sperm production (5–7). Moreover, many experimental and clinical data have highlighted the importance of lipid metabolism in the control of testicular physiology and male fertility (8). As example, the depletion of cholesterol in plasma and tissues in a mouse model deficient for the gene encoding 24-dehydrocholesterol reductase (*Dhcr24*) leads to mouse infertility (9).

## CHOLESTEROL HOMEOSTASIS

Cholesterol is essential for mammalian cell functions and integrity. It is an important structural component maintaining the permeability and fluidity of the cell membrane. Within the cells, the cholesterol homeostasis is tightly regulated to ensure normal cellular processes. Cholesterol homeostasis is strictly regulated at the cellular level.

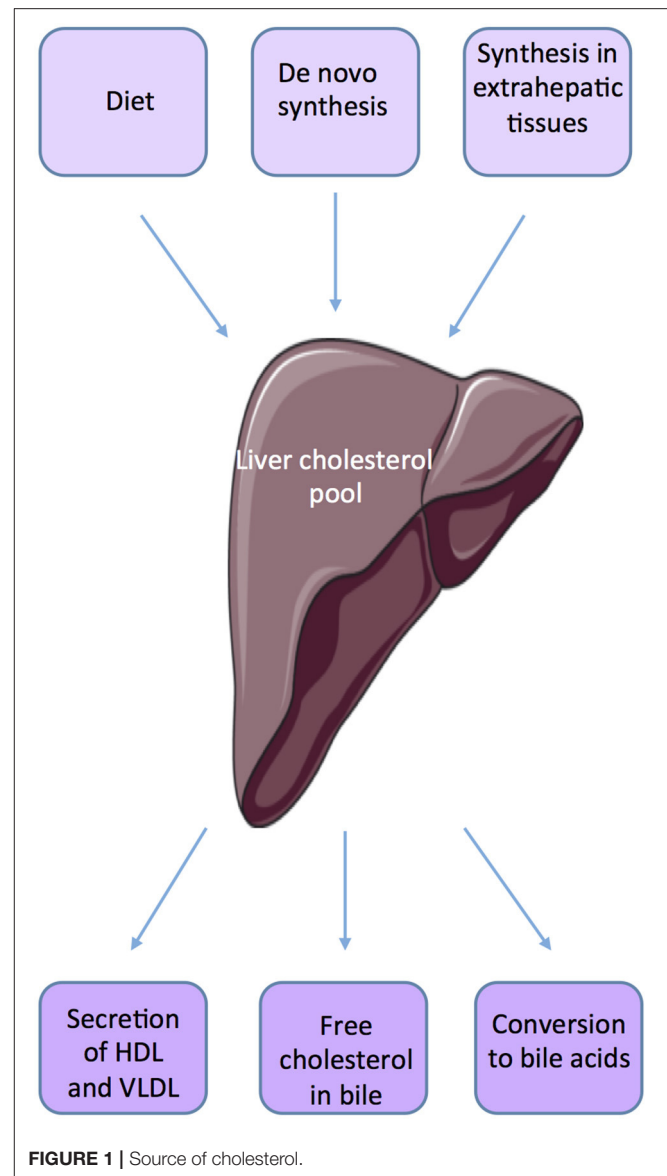
Cholesterol is also a precursor for steroid hormones synthesis such as bile acids and vitamin D. Oxidized derivatives of cholesterol, known as oxysterols, are also implicated in numerous biological processes.

The main source of cholesterol is food. Dietary cholesterol is first routed from the small intestine to the liver, and then redistributed to the requesting organs. Cholesterol is uptake from lipoproteins; the Scavenger receptor B type 1 (SR-B1) is the cell surface high-density lipoprotein (HDL) receptor that mediates HDL-cholesterol ester (HDL-CE) uptake. Next to this, less than half of the cholesterol originates from *de novo* synthesis. Cholesterol synthesis is a multi-step enzymatic process. Synthesis of cholesterol begins from the acetyl-CoA, which is transported from the mitochondria to the cytosol. A series of reactions give rise to Hydroxy-methylglutaryl-CoA (HMG-CoA), which is then converted to mevalonate by HMG-CoA reductase. Finally, multi-steps will then give rise to cholesterol.

The cellular efflux of cholesterol is essential to maintain the homeostasis, as cells in peripheral organs do not express genes involved in the catabolism of cholesterol. The efflux of cholesterol requires acceptors such as HDL. There is either a simple diffusion (aqueous diffusion pathway) or a facilitated one through SR-B1 pathway. There could also be active process *via* members of the ATP-binding cassette (ABC) family transporters such as ABCA1 and ABCG1.

Within the body, the liver plays an important role in maintaining cholesterol homeostasis by regulating absorption and synthesis to prevent over accumulation in the plasma and tissues (10, 11) (**Figure 1**). The small intestine is also an important actor of *de novo* synthesis of cholesterol, bile synthesis, and absorption and fecal excretion. Molecular mechanisms involved have been quite well defined in the organs such as liver, adipose tissue, and intestine (12). The cross talks between liver and intestine illustrate the complex mechanisms involved in the regulation of cholesterol homeostasis through the involvement of several factors such as SREBP-2 (sterol regulatory element-binding protein), LXR (Liver X receptor, NR1H2, NR1H3), and FXR $\alpha$  (Farnesoid X receptor, NR1H4). Many studies suggest that SREBP and LXRs work in a coordinated manner to maintain cholesterol homeostasis (11, 13). SREBP2 up-regulates the expression of genes involved in the biosynthesis and uptake of cholesterol when cholesterol level is low.

LXR $\alpha$  and LXR $\beta$  are ligand-activated nuclear receptors; which are critical for the elimination of excess cholesterol. LXR $\alpha$  is primarily expressed in macrophages, intestine, liver, adipose, and kidney, whereas LXR $\beta$  is more ubiquitous (14). The major endogenous LXRs agonists that have been identified are oxysterols [20(S)-, 22(R)-, 24(S)-, 25-, 27-hydroxycholesterol; 24(S), 25-epoxycholesterol] and cholesterol biosynthetic intermediates [desmosterol, follicular fluid meiosis-activating sterol (FF-MAS), or testis meiosis-activating sterol (T-MAS)] (15, 16). Two synthetic LXR $\alpha/\beta$  agonists were developed and have been used widely such as T0901317 and GW3965 (17). The physiologically most important oxysterols are generated in cells by mitochondrial or endoplasmic reticulum cholesterol hydroxylases belonging to the cytochrome P450 family (CYP46A1, CH25H, CYP27A1 CYP3A4, and CYP7A1). Several natural compounds derived from plants have also been shown to activate (phytosterols, diterpenes...) or inhibit (naringenin) LXRs activity (15). Studies from LXR-knockout mice have demonstrated that LXRs are important in the



**FIGURE 1** | Source of cholesterol.

control of cholesterol homeostasis. Activation of LXRs protects cells from cholesterol accumulation by suppressing cholesterol biosynthesis, activating the conversion of cholesterol to bile acids in the liver, and decreasing intestinal cholesterol absorption (18, 19). Indeed, LXR $\alpha$ -knockout mice fed with a high-cholesterol diet show accumulation of cholesteryl-esters in the liver (20). LXR $\alpha$  is the prevalent isoform acting as hepatic sensor of cholesterol. While *Lxr $\alpha$*  knock-out mice showed an impaired hepatic function when fed high fat diet, mice lacking *Lxr $\beta$*  are able to resist to the deleterious impact of diet enriched in cholesterol (20, 21).

Cholesterol is the precursor for bile acids (BAs) synthesis within the liver. These molecules are the main constituents of bile and ensure solubilisation and emulsification of fat to help digestion (22). Their synthesis and excretion are major mechanisms of cholesterol catabolism in mammals.

Enzymatic modifications of cholesterol result in the production of primary BAs: cholic acid (CA) and chenodeoxycholic (CDCA) (23). There are two main BA synthesis pathways (24). The «classical» pathway involves CYP7A1 and CYP8B1 enzymes while the «alternative» pathway involves the cholesterol 27-hydroxylase CYP27A1 and 25-hydroxycholesterol 7- $\alpha$ -hydroxylase CYP7B1. Before their excretion, BAs are, in part, conjugated with glycine or taurine in the liver. This leads to the production of tauro-, or glyco-conjugates BAs. Stored in the gallbladder, primary BAs and conjugates are discharged during the meal into the duodenum to facilitate fat digestion. In the ileum, BAs are in part deconjugated and modified to give rise to secondary BAs (25). Deoxycholic acid (DCA) and lithocholic acid (LCA) are derived respectively from CA and CDCA. BAs have been described as signaling molecules that signal through two main receptors: the nuclear Farnesoid X receptor  $\alpha$  (FXR $\alpha$ ; NR1H4) (26–28) and the membrane G protein-coupled bile acid receptor TGR5 (GPBAR1) (24, 29).

In addition, the nuclear receptor FXR $\alpha$  plays important role on cholesterol homeostasis through the regulation of bile acid metabolism in the liver and in the intestine. In the liver, FXR reduces the expression of the genes encoding key enzymes of bile acids biosynthesis pathways: the cholesterol 7 $\alpha$ -hydroxylase (Cytochrome P450 7a1; CYP7A1) and the sterol 12 $\alpha$ -hydroxylase (CYP8B1). In the intestine, FXR $\alpha$  activates the expression of the Fibroblast Growth Factor 19 (FGF19 in human) or FGF15 (mouse) which acts in an endocrine manner on hepatocytes *via* the Fibroblast Growth Factor Receptor 4 (FGFR4) to repress *Cyp7a1* expression (30, 31).

## THE TESTIS, CHOLESTEROL HOMEOSTASIS

The testis produces male gametes, namely spermatozoa. It is composed of a network of seminiferous tubules, separated from each other by the interstitial space. These two compartments define the two major functions of the testis with the production of gametes (exocrine function) and sexual hormone (endocrine function). Leydig cells within the interstitial space provide part of the endocrine function, and the exocrine function takes places within the seminiferous tubules. Their efficiencies are based on the specific actions of the different cell types and their interactions.

The purpose of this special issue is to collect recent acquisition on the role of cholesterol and/or derivatives as signaling molecules, whose alteration can determine the onset or progression of different pathologies. Here, we will highlight the roles of pathways activated by cholesterol and derivatives through nuclear receptors on male fertility disorders.

### The Testicular Endocrine Function

In human and rodents, testosterone is the main type of circulating androgens, which is mainly synthesized by the Leydig cells. Testis also produces small amounts of other hormones such as estrogen and progesterone.

Maintenance of an adequate concentration of intra-testicular testosterone is essential for testicular function, especially for spermatogenesis (32). The hypothalamo-pituitary axis exerts a major control on testicular steroidogenesis. Gonadotropin Releasing Hormone (GnRH) secreted by the hypothalamus, stimulates the synthesis and release of Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH) by the gonadotropic cells of the anterior pituitary (33). The LH acts directly on the Leydig cells to control their steroidogenic activity *via* the activation of the luteinizing hormone/choriogonadotropin receptor, LHCGR. In return, sex steroids exert a negative feedback on their own synthesis. LH is involved in the regulation of steroidogenesis at different levels. LH pathway promotes the activation of cholesterol ester hydroxylase increasing the free cholesterol pool (34) and also promotes the transfer of cholesterol from the outer membrane to the inner membrane of the mitochondria. LH signaling pathway leads to the transcriptional activation of LH target genes such as *Star* (35), *Cyp11a1* (36), and *3 $\beta$ Hsd* (37).

The major role of cholesterol in the endocrine function has been well established, as cholesterol is the precursor of steroids. There are multiple sources of cholesterol that contribute to steroidogenesis such as *de novo* synthesized, (b) stored cholesteryl esters, exogenous lipoprotein-supplied cholesterol as well as plasma membrane-derived cholesterol. The plasma lipoproteins are the main source of steroid synthesis. The homeostasis of the interstitial tissue, mainly in Leydig cells, involved factors of cholesterol homeostasis such as HMG-CoA reductase, HSL, and ACAT (38, 39). Among others, the links between cholesterol and steroid synthesis are highlighted by the control of the SCAP/SREBP pathway, a regulator of cholesterol homeostasis by cAMP the secondary messenger of the LH pathways.

Sex steroid hormones share a common biosynthesis pathway, with cholesterol as unique precursor. Leydig cells are able to *de novo* synthesize cholesterol or can also use stored cholesterol ester to ensure steroidogenesis function (40). A recent study shows that the plasma membrane is one of the richest source of free cholesterol available for acute steroidogenesis (41). The first enzymatic step of steroidogenesis involves the conversion of cholesterol to pregnenolone, which occurs within mitochondria. It requires the transport of cholesterol from the outer mitochondrial membrane to the inner membrane. This limiting step is mainly provided by the Steroidogenic Acute Regulatory protein (STAR) (42). This crucial point has been highlighted by the use of transgenic mouse models. Mice lacking *Star* present gonadal insufficiencies due to steroidogenesis defects associated with lipid accumulation in the interstitial compartment (43). Recently, using a mitochondrial reconstitution assay system, it has been shown that SNARE proteins (Soluble N-ethylmaleimide-sensitive-factor Attachment protein REceptor) are vital components contributing to cholesterol transport within the mitochondria (44). Then, the biosynthesis involves the oxidative cleavage of the side-chain of cholesterol by cholesterol side-chain cleavage enzyme (cytochrome P450<sub>scc</sub>, CYP11A1), a mitochondrial cytochrome P450 oxidase, to give pregnenolone (45). Pregnenolone may be converted to testosterone through two pathways called

Delta 4 ( $\Delta 4$ ) and Delta 5 ( $\Delta 5$ ) (46). The Delta 4 ( $\Delta 4$ ) pathway involves conversion of pregnenolone to progesterone by the action of the enzyme 3 $\beta$ -Hydroxysteroid Dehydrogenase (3 $\beta$ HSD). Progesterone is then metabolized by cytochrome P450 17 $\alpha$ -hydroxylase/17,20 lyase (CYP17A1) in androstenedione, substrate of the 17 $\beta$ -hydroxysteroid dehydrogenase enzyme (17 $\beta$ HSD) for testosterone synthesis. The Delta 5 ( $\Delta 5$ ) pathway is initiated by the CYP17A1 enzyme, that allows the conversion of pregnenolone to dehydroepiandrosterone (DHEA). Then, 17 $\beta$ HSD and 3 $\beta$ HSD enzymes ensure its conversion into androstenediol or androstenedione, and testosterone. The 3 $\beta$ HSD enzyme allows switching from  $\Delta 5$  to  $\Delta 4$  pathway. In primates including humans, the  $\Delta 5$  pathway predominates because CYP17A1 enzyme has a low activity 17, 20 lyase for the conversion of 17 $\alpha$ -hydroxyprogesterone to androstenedione (47, 48). A recent proteomic analysis of murine testis lipid droplets (LDs) revealed that these LDs contained a large number of enzymes involved in metabolism and steroidogenesis. Testicular LDs could be an active organelle functionally involved in steroidogenesis (49).

Androgens exert autocrine action on Leydig cells via the androgen receptor (AR) to control their own synthesis. The AR knockout mice in Leydig cells have decreased plasma testosterone concentrations, despite high rates of LH. This hypoandrogenicity is explained by a defect in the expression of key genes encoding steroidogenic enzymes *Cyp17a1*, *3 $\beta$ hsd*, and *17 $\beta$ hsd*. This was shown to be associated with an arrest of spermatogenesis at the stage of round spermatid causing infertility (50, 51). In humans, mutations on the gene encoding the receptor of androgen AR (*NR3C4*) result in androgen resistance syndrome (52). Estrogen action is also important in regulating testicular function. Estrogen mainly mediated their signal through the activation of two specific estrogen receptors (ER), ER $\alpha$  and ER $\beta$  (53, 54). Estrogen effects on steroidogenesis have been elucidated using mouse model. It has been shown that ER $\alpha$  regulates fetal and neonatal steroidogenesis and ER $\beta$  is implicated in the control of neonatal gametogenesis. *Era* is expressed in Leydig cells during fetal and neonatal life and its deletion increases testosterone production during fetal life from 13.5 dpc (55). In human, estrogens also play a crucial role in the control of reproductive functions. Studies in patients with inactivating mutations of ER $\alpha$  or *CYP19* (aromatase cytochrome P450) suggest a role of estrogens signaling in human male fertility (56). Next to the steroid receptors, it has been shown that numerous nuclear receptors are implicated in the regulation of steroids synthesis. This was recently well reviewed (57, 58). Nuclear receptor family has been described to locally control the expression and activity of enzymes involved in steroidogenesis. Among these, the Steroidogenic Factor 1 (SF-1) and the Liver Receptor Homolog 1 (LRH-1) are positive regulators of hormone steroids synthesis. They stimulate the expression of common target genes such as *Star*, *Cyp11a1*, *Cyp17a1*, and *3 $\beta$ hsd* encoding enzymes of steroidogenesis (59–62). SF-1 and LRH-1 are also involved in the regulation of cholesterol biosynthesis by regulating the expression of *Sr-b1* (scavenger receptor class B type I) gene which provides plasma cholesterol to steroidogenic tissues (63, 64), and the

enzyme Hydroxymethylglutaryl-CoA synthase and reductase (HMG CoA) involved in the biosynthesis of cholesterol (65, 66).

In addition, it has been shown that LXR $\alpha$  is expressed within Leydig cells (8, 67, 68). It was demonstrated that in 2.5-month-old LXR $\alpha$ -deficient mice, testosterone production is significantly lower than in wild type control mice. This was associated with a decrease of the expression of *3 $\beta$ hsd* enzyme (67). Interestingly, LXR agonist T0901317 increased testosterone concentration in wild-type mice in association with an accumulation of StAR at both mRNA and protein levels (67).

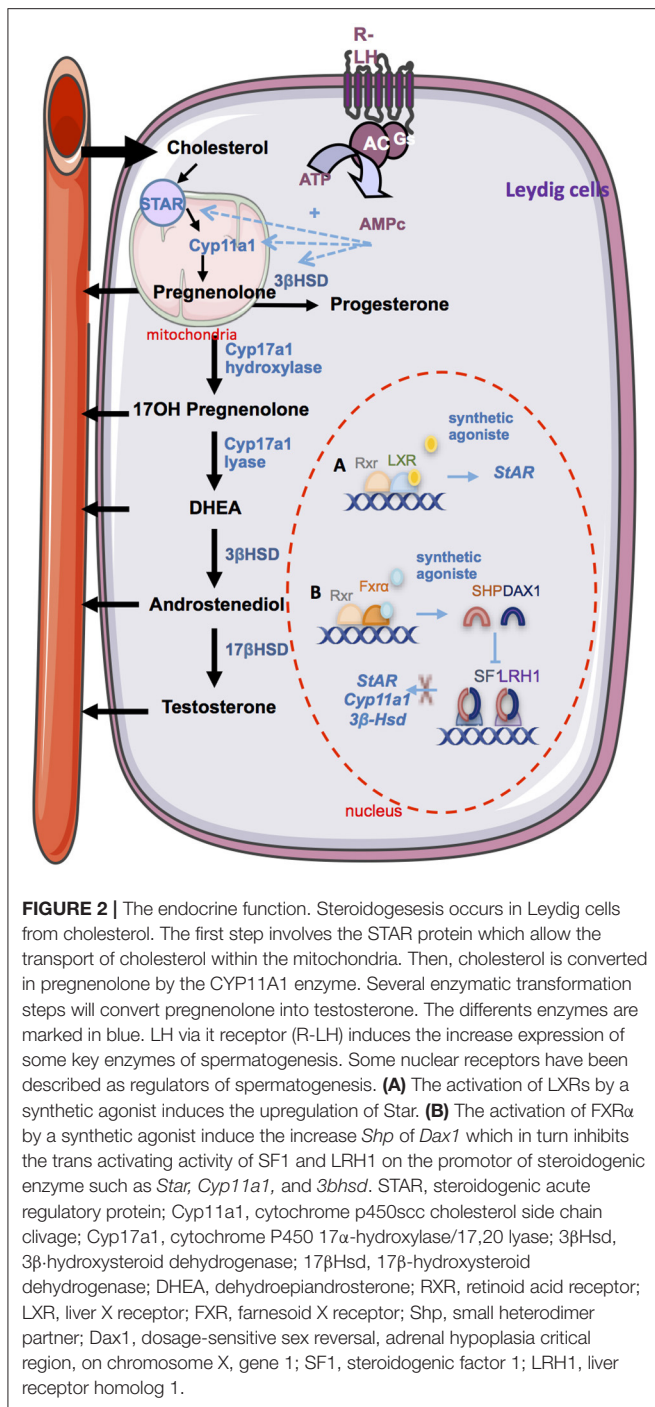
Next to this, a negative regulation of steroid synthesis involving receptors of bile acids has been defined as an important regulator of testicular physiology. BAs receptors, FXR $\alpha$  and TGR5, are expressed within the testis (69, 70) and have been recently shown to be implicated in testis dysfunction (71). Detectable levels of bile acids have been measured in the testis of mice in normal physiological conditions (72) and recently, it was shown that testis expresses key enzymes of BAs synthesis and is able to produce BAs (73).

The bile acids nuclear receptor FXR $\alpha$  is implicated in steroidogenesis regulation. In 2007, the presence of FXR $\alpha$  within mouse Leydig cells was demonstrated for the first time (69). While *Fxr $\alpha$ <sup>-/-</sup>* male presented the same plasma testosterone level than control mice, induction of FXR $\alpha$  with a synthetic agonist in wild type mice results in a decrease of androgen production independently of the hypothalamo-pituitary axis. The activation of the FXR $\alpha$  receptor in Leydig cells induces transcription of the gene encoding the orphan nuclear receptors SHP (Small Heterodimer Partner, *Nr0b2*) and DAX-1 (dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1, *Nr0b1*), two orphan nuclear receptors, which are negative regulators of steroidogenic Leydig cells activity. Indeed, SHP AND DAX-1 inhibit the expression of *Lrh1* and *Sf1*. SHP also interacts with LRH1 thereby inhibiting its trans-activating activity. This decreases expressions of genes encoding key enzymes of steroidogenesis and thus leads to lower testosterone levels (69). In that line, through the regulation of the endocrine function, it has been demonstrated that BA-FXR $\alpha$  pathways also regulate male sexual maturation (74). Mice fed a diet supplemented with BAs during pubertal age show increased incidence of infertility. This is associated with altered differentiation and increase apoptosis of germ cells due to lower testosterone levels. This is sustained by the fact that supplementation with testosterone abolished the effect of CA-diet on germ cell apoptosis.

These overall data clearly suggest a complex regulation of testicular steroidogenesis by nuclear receptors whose activities are regulated by cholesterol derivatives such as oxysterols and bile acids (Figure 2).

## The Testicular Exocrine Function

Germ cell differentiation is closely related to somatic Sertoli cells functions, which provide structural and nutritional supports (Figure 3).

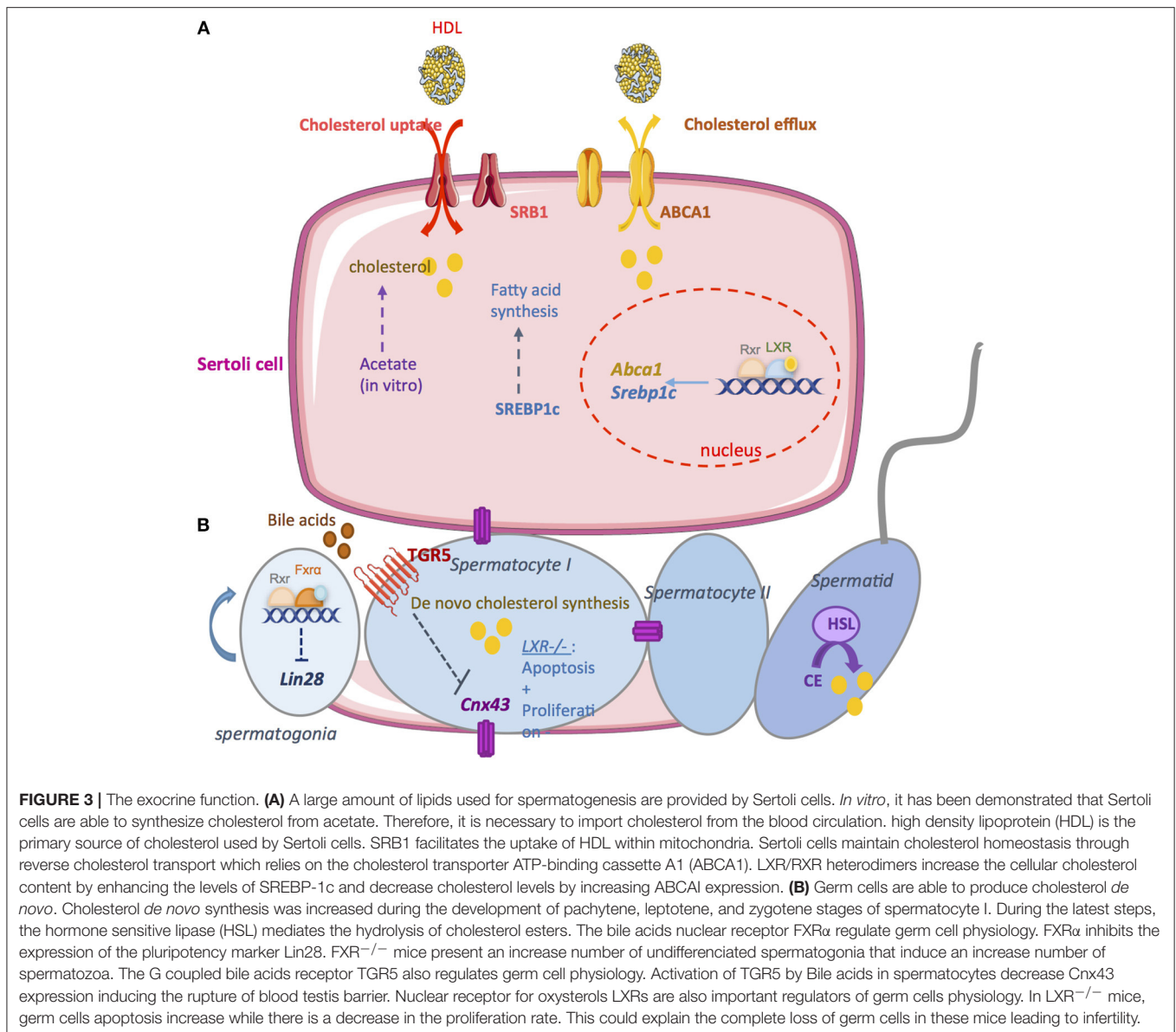


### The Sertoli Cells

One primary function of Sertoli cells is to set up the blood testis barrier (BTB) at puberty under the action of androgens, retinoids and thyroid hormones (75–77). The BTB is composed of specialized junctions established between adjacent Sertoli cells near the basement membrane of seminiferous tubules. That defines Sertoli cells polarity giving them different structural and functional poles. The BTB divides the seminiferous tubules in basal and apical compartments. Self-renewal and differentiation

of spermatogonia as well as progression to the stage pre-leptotene spermatocyte I occur in the basal compartment. The two meiotic divisions and post-meiotic steps take place in the apical compartment. The BTB regulates and restricts the passage of nutritional substances, vital molecules, and toxic compounds in the apical compartment of the seminiferous tubules. BTB also defines an immune privileged environment. It isolates many antigens present on the surface of post-meiotic germ cells from the systemic circulation thus preventing the emergence of the corresponding antibodies and autoimmune diseases leading to infertility (78). Sertoli cells also participate to the secretion of components of the extracellular matrix necessary to maintain the integrity of the seminiferous epithelium and intercellular junctions (79). In addition, they synthesize proteases and protease inhibitors contributing to the dynamic of junction complexes allowing the migration of germ cells (80). Sertoli cells provide a set of essential nutrients for germ cells survival and differentiation, including amino acids, carbohydrates, lipids and vitamins (80). Sertoli cells are able to metabolize glucose into pyruvate and lactate (81) which is the preferential energy substrate for post-meiotic germ cells to support their metabolic activity. Sertoli cells secrete numerous growth factors modulating the proliferation and/or differentiation of germ cells (82). In addition Sertoli cells secrete several transport proteins that will ensure the passage of metal ions (Transferrin) (83) or hormone (androgen-binding protein, retinoid binding protein) (84) through the BTB for the metabolic requirement of the post-meiotic germ cells.

Sertoli cells provide large amounts of lipids used for spermatogenesis. *In vitro*, it has been demonstrated that Sertoli cells are able to synthesize cholesterol from acetate (85). However, this *de novo* synthesis is not a sufficient source of cholesterol to ensure spermatogenesis *in vivo*. Therefore, it is necessary to import cholesterol from the blood circulation. In rodent, it has been demonstrated that high density lipoprotein (HDL) is the primary source of cholesterol used by Sertoli cells (86). The high-density lipoprotein transporter SR-B1 is essential to import cholesterol from blood. SR-B1 is an integral membrane glycoprotein expressed in Leydig and Sertoli cells (87, 88). This transporter facilitates the uptake of HDL. It has been demonstrated that the overexpression of SR-B1 is associated with an increase of esterified cholesterol in Sertoli cells (89). SR-B1 is also implicated in the phagocytosis of apoptotic germ cells. This may also increase cholesterol levels in Sertoli cells (90). Another important point is the elimination of cholesterol overflow. Cholesterol is esterified and stored in lipid droplets to limit toxicity. Sertoli cells maintain cholesterol homeostasis through reverse cholesterol transport. This reverse transport primarily relies on the cholesterol transporter ATP-binding cassette A1 (ABCA1) (91). Genetic mutations in *ABCA1* lead to the absence of HDL in the plasma and the accumulation of esterified cholesterol in tissues, increasing the incidence of atherosclerosis (92). *ABCA1* is highly expressed in the testis and it was observed that within the seminiferous tubules, *ABCA1* is more expressed in Sertoli cells (91). Authors demonstrated that TM4 Sertoli cells lacking *Abca1* or primary Sertoli cells cultured from *Abca1*<sup>-/-</sup> mice fail to efflux cholesterol. *In vivo*, *Abca1*<sup>-/-</sup>



mice present lipid accumulation in Sertoli. At 6 months, intra-testicular testosterone levels and sperm counts are significantly reduced in *Abca1* $^{-/-}$  mice compared with controls. Thereby, the fertility of *Abca1* $^{-/-}$  mice is reduced across their reproductive lifespans. These results indicate that ABCA1 play important role to Sertoli cells function and male fertility.

Regarding the cellular signaling, it has been demonstrated that LXR $\beta$  is expressed in Sertoli cells. The *Lxr $\beta$*  $^{-/-}$  knockout mice are fertile with few observed defects (57, 67). However, these mice present cholesteryl ester accumulation in the Sertoli cells. This is associated with a decrease of germ cell proliferation rate. Interestingly, it was shown that men with azoospermia present a significant decrease of *Lxr $\beta$*  expression within the testis associated with fewer proliferating germ cells (93). Mice deficient for both LXRs isoforms present progressive

testicular degeneration associated with germ cell depletion. *Lxr $\alpha$*  $^{-/-}$ ; *Lxr $\beta$*  $^{-/-}$  are reported to be infertile by 7–9 months of age (67, 68). As in *Lxr $\beta$*  $^{-/-}$  mice, lipid accumulation in Sertoli cells appears to be the earliest phenotype of *Lxr $\alpha$*  $^{-/-}$ ; *Lxr $\beta$*  $^{-/-}$  mice. A recent study further investigate the roles of LXR $\beta$  in Sertoli cells by generating a mouse model that re-expresses *Lxr $\beta$*  only in Sertoli cells in the *Lxr $\alpha$*  $^{-/-}$ ; *Lxr $\beta$*  $^{-/-}$  mice (94). Authors showed that this *Lxr $\beta$*  re-expression is fundamental for Sertoli cells to maintain functional BTB and to sustain the germ cell pool. As expected, *Lxr $\beta$*  re-expression restored lipid homeostasis in Sertoli cells and regulates the endocrine function of Leydig cells. However, these mice are infertile with spermatogenesis defects. Furthermore, it was also demonstrated that wild-type animals exposed to the synthetic LXR agonist T0901317, within diet, exhibited increased levels of the LXR target genes sterol

regulatory element-binding protein-1c (SREBP-1c) and ABCA1 (14, 95). Another study comforts the previous results in MSC-1 cells treated with T0901317 where ABCA1 levels were also increased (68).

These data clearly demonstrate the main roles of LXRs in the regulation lipid homeostasis of Sertoli cell and thus male fertility.

### The Germ Cell Lineage

Spermatogenesis is a highly coordinated process leading to the production of male haploid gametes differentiated from diploid germ stem cells. Spermatogenesis begins with a proliferative phase with the differentiation of type A spermatogonia located in contact with the basement membrane of the seminiferous tubules. A<sub>s</sub> (A single) spermatogonia possess the ability to renew the population of undifferentiated germ cells. Spermatogonia can also enter in the differentiation process. Then spermatogonia engaged in meiosis, the diploid spermatocyte gives rise to haploid cells called secondary spermatocyte or spermatocyte II and then round spermatids. Meiosis step is followed by spermiogenesis; which is the terminal process of spermatogenesis differentiation. The spermatids nucleus will be reorganized leading to chromatin compaction (96). Firstly the majority of histones is replaced by small basic transition proteins (TNP1 and TNP2). Secondly, transition proteins will be replaced by specific testis nuclear protein, protamines (PRM1 and PRM2) (97) to protect the genome from physical and chemical attack.

There is an intimate association between cholesterol metabolism and fertility during spermatogenesis. Cholesterol is required for the mass production of germ cells during spermatogenesis. A study determined that cholesterol *de novo* synthesis was increased during the development of pachytene, leptotene, and zygotene stages (98), which was associated with increased diameter and surface area of germ cells. In later stages, the rate of cholesterol synthesis tends to decrease and remains low throughout the following phases of spermatogenesis. During the last steps of spermatogenesis the Hormone-sensitive lipase (HSL) is known to mediate the hydrolysis of cholesterol esters. It has been shown that the HSL is localized in elongating spermatids and spermatozoa. The most striking phenotype of *Hsl*<sup>-/-</sup> mice is male sterility caused by oligospermia (99). *Hsl*<sup>-/-</sup> mice were found to accumulate diacylglycerols and cholesterol esters in the testis. A recent study further investigate *Hsl*<sup>-/-</sup> mice testis. Authors showed that spermatogenesis is arrested just before spermiogenesis elongation step (100). An important pathway of lipid metabolism must be lost in *Hsl*<sup>-/-</sup> testes causing an arrest of cell differentiation.

Like any animal cell, spermatozoa have a lipid bilayer plasma membrane. The proportion of the different constituents gives to the spermatic plasma membrane unique properties. The spermatic membrane has been shown to be rich in polyunsaturated fatty acids (PUFAs) important for ensuring the viability and mobility of the spermatozoon (101–103). The high proportion of PUFA also seems to play an important role in the process of membrane fusion between spermatozoon and oocyte. Indeed, PUFAs contribute to flexibility and

membrane fluidity (104, 105). The high proportion of PUFA, especially docosahexaenoic acid (DHA) in humans, is important for fertility. Indeed, one study showed a decrease in PUFAs and an increase in saturated fatty acids (SFA) in spermatozoa of asthenozoospermic men compared to sperm of normozoospermic men (106). Another study also found a negative correlation between body mass index and DHA and a positive correlation between DHA and normal sperm parameters (107). Sterols are the second major components of the plasma membrane. Cholesterol is the most abundant sterol found in the spermatic membrane of many species (102). Spermatozoa are enriched in cholesterol within the seminiferous tubules. Indeed, in the spermatocyte stage, the germ cells are able to synthesize *de novo* cholesterol to allow the increase of their membrane surface (98). Sertoli cells also participate in this process by providing to sperm cells *de novo* synthesized cholesterol or derived from the bloodstream (85, 89). More recently, the presence of oxidized cholesterol derivatives has been described in several species including mice (108). These oxysterols could influence the membrane fluidity (109). The spermatic membrane is also composed of complex lipids such as phospholipids (PL) and sphingolipids (77, 85). The lipid composition of spermatozoa is of great importance during capacitation in the female genital tract and also in membrane fusion events during fertilization. After spermiation, the lipid composition of the spermatic membrane is not definitive and will undergo many modifications during the epididymal transit and the capacitation process. Lipids largely influence post-testicular maturation of spermatozoa. During the epididymal transit, the lipid composition of the gamete is strongly reshuffled (110–112). These modifications influence the membrane dynamics in order to confer greater membrane fluidity. These lipid rearrangements are finely regulated. The epididymis produces lipid vesicles allowing protein transfer to spermatozoa. Finally, capacitation is a stage of post-testicular maturation partly regulated by the lipid composition of the spermatic membrane (113). Therefore, regulation of the lipid profile of gametes is essential for maintaining male fertility. It has recently been shown that the lipid composition of spermatozoa varies according to the dietary intake of lipids (114), demonstrating a direct correlation between diet and gamete composition.

Mammalian spermatozoa are specialized and polarized cells, with one specific function which is the fertilization of the female oocyte. This fertilizing capacity is acquired during a multistep process after spermiation called post testicular maturation. Spermatozoa maturation starts during its epididymal transit and ends in the female genital tract. The maturation of spermatozoa remains the major function of the epididymis. Epididymal maturation is essential for the acquisition of fertilizing power but is also essential to ensure normal embryonic development (115). Epididymal maturation consists of morphological, functional, and biochemical changes.

Regarding the signaling pathways involved, the LXR signaling pathways control implicated in the regulation of spermatogenesis (67). Spermatogenesis results from a balance between cell

proliferation, cell differentiation, and cell death. Proliferation and apoptosis are respectively altered in *Lxr $\beta$ <sup>-/-</sup>* and *Lxr $\alpha$ <sup>-/-</sup>* mice, whereas these mice did not show any fertility troubles. In the *Lxr $\beta$ <sup>-/-</sup>* mice the lower proliferation rate was compensated by a decreased apoptosis rate and in *Lxr $\alpha$ <sup>-/-</sup>* mice the higher apoptosis level was associated with an increased proliferation rate. The lack of both LXRs led to a dramatic decline in proliferation and increase in apoptosis. This could explain the complete loss of germ cells in these mice and thus infertility. Recently, the implication of LXRs in the physiopathology of defective spermatogenesis in non-obstructive azoospermia (NOA) patients was investigated (93). Indeed, abnormalities observed in NOA patients look like those observed in *Lxr<sup>-/-</sup>* mice. It has been shown that *Lxr* mRNA levels were decreased in NOA specimens and positively correlated with germ cell number. As expected, authors showed a decreased level of *Idol* (Inducible Degradator of the LDL receptor) and *Srebp1c* (Sterol regulatory element-binding protein 1), which are LXR target genes.

Next to this, a recent study has shown that the G coupled bile acids receptor TGR5 was expressed within the testis (70). It has been shown that BA-TGR5 signaling pathways alter testicular epithelium integrity leading to adult male fertility disorders (72). Indeed, after 4 months of dietary cholic acid (CA) supplementation, 25% of male mice were sterile and in fertile ones a 20% decrease of pups per litter was reported. Male exposed to BAs presented lower spermatozoa in the tail of the epididymis explaining the fertility defect. Interestingly, authors showed that *Tgr5<sup>-/-</sup>* mice were preserved of the CA deleterious effects on the testis. At the molecular level, BAs exposure induced a repression of cell-cell interactions network through the down-regulation of N-cadherin as well as connexin 43 (CX43) expression. This leads to germ cell sloughing, rupture of the BTB and then spermatids apoptosis. In humans, a reduction in the expression of CX43 was shown to be associated with the degree of spermatogenesis defect (116–118).

Recently, it has been shown that bile acids nuclear receptor FXR $\alpha$  regulates germ cell physiology (73). Authors showed that FXR $\alpha$  plays a role in the establishment and maintenance of the undifferentiated germ cell pool that in turn influences male fertility. *Fxr $\alpha$ <sup>-/-</sup>* males present an extended fertility with aging. In *Fxr $\alpha$ <sup>-/-</sup>* males, the maintenance of fertility capacities results from a higher number of undifferentiated germ cells during aging associated with high spermatozoa production. FXR $\alpha$  regulates the expression of several pluripotency factors within the germ cell lineage. *In vitro* approaches show that FXR $\alpha$  controls the expression of the pluripotency marker Lin28 in the germ cells.

## Obesity and Male Fertility

In the last decades infertility has become a global public health issue affecting 15% of all reproductive couples. It has been estimated that 70 million couples worldwide experience subfertility or infertility (119). Male factors are responsible for ~25% of cases of infertility (2). Currently, the etiology of low semen quality is poorly understood. Many physiological, environmental and genetic factors have been implicated (120).

Metabolic syndrome (MetS) is a group of risk factors such as high blood pressure, high blood sugar, unhealthy cholesterol levels, and abdominal fat. The association between MetS and male hypogonadism is well established and reviewed (121). Cross-sectional studies have found that between 20 and 64% of obese men have low total testosterone levels (122). A number of studies also show that low testosterone is associated with insulin resistance and an increased risk for diabetes mellitus and MetS in men. Testosterone supplementation seems to be beneficial on inflammation, muscle mass, lipid profile, sexual function, and improves comorbidities of obesity, MetS, or cardiovascular disease in males (121).

Evidence from human studies indicates that male obesity and components of the diet could play an important role in the deregulation of spermatogenesis, sperm maturation, or fertilizing ability. Obese or overweight men could present a decrease sperm quantity, quality, and motility (123, 124). A study showed differences between obese and lean patients regarding the acrosome reaction (125). Sperm acrosome reaction is impaired in obese men associated with altered circulating levels of estradiol and sperm cholesterol content. Obese men can also present an increase sperm DNA damage or lower embryo implantation rate (122, 126). The majority of studies in humans focuses on the impact of obesity on male fertility and do not take into account the plasma lipid profile. It is reported that 65% of infertile men show hypercholesterolemia and/or triglyceridemia (127). The impact of dyslipidemia on male fertility remains controversial. Even if cholesterol and lipid homeostasis is essential for male fecundity (57, 128), a recent study reported that hypercholesterolemia is not associated with sperm concentration or motility in men (129). It has also been shown that there is no correlation between sperm concentration and serum total cholesterol or triglyceride in human (130).

Some animal studies revealed that high cholesterol diet can impair fertility and lower sperm quality. Rabbit fed a High fat diet (HFD) is an experimental model of metabolic syndrome (MetS) that closely similar to the human syndrome (131–133). This model has been characterized by hyperglycaemia, hypercholesterolemia, hypertension, hypogonadotropic hypogonadism, penile alterations (131, 132, 134) or nonalcoholic steatohepatitis. This model is very useful to study the deleterious impact of MetS symptoms such as hypercholesterolemia on male fertility. It has been demonstrated that supplementation with testosterone (131, 132) with the FXR $\alpha$  agonist the obeticholic acid (133, 134) normalize several MetS symptoms including HFD-induced penile alterations. These animals presented abnormal sperm morphology, decreased sperm number and declined percentage of motile sperm or acrosome reaction (134–136, 138, 139). It has been shown that administration of tamoxifen, used in the treatment of idiopathic male infertility to HFD rabbits partially restored sperm motility, but further decreased morphology and increased spontaneous acrosome reaction (136). It has also been shown that hypercholesterolemic rabbits present lower testicular efficiency related to both a decrease in spermatogonial cells and an increase in germ cell apoptosis (137). On the other hand,



spermiogenesis, which is the last step of spermatogenesis, was also affected in these animals. In post-meiotic germ cells from hyper-cholesterolemic rabbits, abnormal development of acrosome, nucleus, and inaccurate tail implantation were associated with actin- $\alpha$ -tubulin-GM1 sphingolipid altered distribution. It was recently shown in rabbits that a cholesterol-enriched diet increased lipid deposition in the seminiferous tubules and disrupted the BTB (138). Total protein levels of the tight-junction protein 1 (ZO-1) and occludin and their distribution patterns were markedly affected, impairing fertility.

A similar study has demonstrated a disruption of BTB in mice exposed to High Fat Diet (HFD) (139). It has been reported that male rodents exposed to HFD present a decreased number of motile spermatozoa associated with a decline of fertilization rates (140–142). Intracellular reactive oxygen species (ROS) and sperm DNA damage were elevated in the HFD group compared with controls that could explain the decrease sperm motility (141). It has been demonstrated that HFD induces a decrease in testosterone production associated with a decrease level of steroidogenic enzyme (143, 144). A recent study suggests that the increase of autophagy process could be a cause of fertility defects induced by HFD (145). Authors demonstrate that inhibiting autophagy with chloroquine improves the decreased fertility of HFD male mice. Furthermore, the excessive activation of autophagy was also observed in sperm samples from obese, sub-fertile male patients.

In addition, some studies were interested in the impact of paternal or maternal exposure to HFD on male offspring (146, 147). It has been shown that preconception exposure of fathers to HFD impairs the motility of spermatozoa of male offspring, despite their control diet consumption (146). Feeding females with a HFD through pregnancy and lactation induces a decrease on LH and testosterone levels in male offspring (147). If translatable to human health, these studies suggest that reproductive defects may be amplified throughout generations because of calorie dense diet.

Interestingly, in the last decade, it has been proposed that derivatives of bile acids (BAs) could be interesting molecules for the treatment of metabolic diseases such as diabetes or obesity. Regarding the links between BA signaling pathways and male testicular physiology (72–74), a study has investigated the consequences of a long term exposure to molecules that activate BA signaling pathways to treat obesity. For that purpose, mice were fed HFD to induce obesity and concomitantly treat with BAs (148). Even if BAs improves abnormalities induced by the HFD such as body weight, glycemia, or lipidic profiles, co-exposure of HFD and BAs leads to a higher level of infertility. This was associated with altered germ cell proliferation, default of endocrine function and abnormalities in cell-cell interactions within the seminiferous tubule.

## CONCLUSIONS

Male fertility disorders represent serious health problem and in 30% of the cases the cause remains unknown. Understanding

the various pathologic mechanisms causing male infertility represents an active area of research. As reviewed here, cholesterol homeostasis is crucial for testicular functions such as steroidogenesis, Sertoli cells function, or germ cell differentiation. Thus, altered concentrations of plasma cholesterol can affect the reproductive function leading to male infertility. This link between lipid homeostasis and male fertility disorders is clearly evident in patients suffering from hyperlipidemia or metabolic syndrome. Lipid-lowering drugs could probably ameliorate some reproductive symptoms. However, epidemiological data are currently scarce and controversial.

All these data could help in proposing new molecular markers to diagnose cases of infertility or to define whether the results obtained on the incriminated nuclear receptors LXR $\alpha$  and FXR $\alpha$  which can be modulated by ligands can lead to concepts transferable to the clinic either to develop new strategies to treat infertile men or new approaches for male contraception. Such perspectives will require the development of testicular-specific LXR or FXR modulators to treat testicular disorders and therefore male disorders without causing their impacts that may lead to metabolic disorders.

In the opposite way, modulating some of these receptors through the use of agonist or antagonist molecules would improve the symptoms of metabolic syndrome diseases. However, it has been shown in mice that modulating nuclear receptors signaling induce deleterious effects on testicular physiology leading to infertility. In that line, as molecules modulating the BA receptor signaling pathways have been proposed to treat metabolic diseases, it will be important to define the molecular mechanisms involved in the deleterious impact of BAs on testis physiology. Such studies will be essential to define how to pharmacologically or genetically modulate bile acid receptors to treat metabolic disorders while minimizing impacts on male reproductive functions. In addition, the identification of the crosstalk between increased BA levels and metabolic diseases on fertility capacities of male mice should be a mechanism explaining rare clinical situations of infertility following bariatric surgical approach in some obese men.

It will be interesting in the future to identify at the clinical levels, using biopsies of patients suffering from liver disorders (cholestasis, hepatitis...), the potential involvement of bile acid signaling pathways in the human regulation of male fertility. According to previous published data, it will be necessary to analyse both BA levels as well as the BA pool composition. Indeed, it appears that secondary BA, namely DCA, is the one whose levels are inversely correlated with fertility. Such analyses on human cohort will be useful to define if BA levels or pool composition might be a useful biomarker linking liver disorders and male infertility; or even if BA could be markers of idiopathic infertility.

In the recent decades, assisted medical procreation (AMP) has been importantly developed to overpass the fertility disorders. Next to *In vitro* fecundation, important efforts have been done in order to develop approach of *in vitro* production of germ cells from spermatogonial stem cells. This strategy is important to propose AMP to azoospermic men. The recent data regarding the role of FXR $\alpha$  in the establishment of the

pool of undifferentiated spermatogonia, suggest that FXRalpha modulators might be useful in such approaches to increase the rate and efficiency to produce male gametes.

## AUTHOR CONTRIBUTIONS

LS, LT, SM, MG, FC, JML, CB and DV have participated to the writing of this review.

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