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INVITED REVIEW

Sperm Biology

Posttesticular sperm maturation, infertility, and hypercholesterolemia

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Cholesterol is a key molecule in the mammalian physiology of especial particular importance for the reproductive system as it is the common precursor for steroid hormone synthesis. Cholesterol is also a recognized modulator of sperm functions, not only at the level of gametogenesis. Cholesterol homeostasis regulation is crucial for posttesticular sperm maturation, and imbalanced cholesterol levels may particularly affect these posttesticular events. Metabolic lipid disorders (dyslipidemia) affect male fertility but are most of the time studied from the angle of endocrine/testicular consequences. This review will focus on the deleterious effects of a particular dyslipidemia, *i.e.,* hypercholesterolemia, on posttesticular maturation of mammalian spermatozoa.

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INTRODUCTION

Mammalian spermatozoa are very specialized and polarized cells, with a specific function: fertilizing their female counterpart, the oocyte. The capacity to fertilize an oocyte is acquired during a long-lasting multi-step process that can be defined as "posttesticular maturation." This maturation, enabling an infertile testicular spermatozoon to become fertile, starts during its epididymal transit[1] and ends in the upper female genital tract, in the vicinity of the oocyte, during the capacitation process.[2,3] During posttesticular maturation events, the modifications of sperm plasma membrane composition and dynamics are essential for fertility,[4,5] and cholesterol plays a crucial role. The importance of epididymal cholesterol homeostasis has been recently reviewed,[6] and this epididymal maturation process prepares sperm cells for the next important maturation step, capacitation, which is also highly dependent on the plasma membrane cholesterol content of the male gametes.[7–9] Even though the central role of cholesterol in male fertility has been known for a long time, few studies have been conducted on the impact of cholesterol metabolism impairment on posttesticular maturation, the emphasis of most being put on the endocrine effects.

Dysregulation of lipid metabolism (dyslipidemia) is increasingly present in occidental societies, with pathophysiological conditions such as obesity, metabolic syndrome and cardiovascular diseases (World Health Organization). Hypercholesterolemia is highly represented among dyslipidemic patients: over 30 million Americans older than 20 years have a high blood cholesterol level (>240 mg dL^{-1}, American Heart Association Statistical Fact sheet 2013). Another study has shown that a high proportion of men at the age for their partners to conceive (45.3% of men aged 30–54 years) were dyslipidemic without taking any treatment.[10] The influence of lipid metabolic disorders on fertility has now become a clinical problem[11,12] and this review will present the state-of-the-art on the impact hypercholesterolemia can have on male fertility, focusing on posttesticular maturation events and sperm functions.

POSTTESTICULAR MATURATION AND CHOLESTEROL

The posttesticular maturation process particularly concerns membrane composition of gametes and begins in the epididymis. It involves remodeling of membrane proteins (relocalization, addition[13,14]) and changes in the lipid composition, in particular of phospholipids and sterols. Changes in the sperm cholesterol content during epididymal transit have been studied in several mammalian species,[6] and a significant decrease of approximately 50% has been found in the ram,[15] rat,[16] hamster,[17] and mouse.[18] The cholesterol loss causes a decrease in the cholesterol/phospholipid ratio, a membrane fluidity indicator, showing that sperm cells increase their membrane fluidity during epididymal transit, which facilitates the subsequent events of capacitation and membrane fusion during fertilization. The distribution of cholesterol in the membrane also appears to be important because the plasma membrane of spermatozoa shows abundant cholesterol-enriched microdomains (lipid rafts), the real organizing centers of cell signaling.[19] These lipid rafts modify the local membrane dynamics and are essential for the regionalized transfer of proteins to the male gamete during epididymal maturation. Indeed, a study in bulls showed that the zona pellucida recognition (P25b) protein was specifically transferred to the sperm lipid rafts *via* lipid rafts found on small lipid vesicles (epididymosomes) emitted by the epididymal epithelium.[20] Moreover, three different subtypes of lipid rafts have been previously characterized in mouse sperm cells, each with a different protein profile as

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determined by proteomic analysis.[21] Many of these proteins are acquired by sperm cells during epididymal transit, showing the importance of the lipid composition of gametes for the proper acquisition and localization of proteins during posttesticular maturation.

The next posttesticular maturation step occurs in the female genital tract during the capacitation process. The lipid composition of sperm cells issuing from the epididymis is crucial in most species, as cholesterol efflux from the plasma membrane is the early event triggering capacitation. The consecutive decrease of the cholesterol/phospholipid ratio modifies plasma membrane dynamics,[7,22] thereby increasing membrane permeability to bicarbonate and calcium ions,[23,24] allowing activation of a soluble adenylyl cyclase (sAC,[25]) and increase in cAMP production. The Ser/Thr kinase PKA (protein kinase A) is also activated, resulting in the initiation of a signaling cascade.[26,27] PKA phosphorylates, among other proteins, SRC kinase, leading to phosphorylation of specific tyrosine residues in sperm proteins.[28] Although phosphorylation of tyrosine residues of proteins during capacitation is well described today, the roles of these proteins in the capacitation process remain poorly understood.[9] Ultimately, with capacitation gametes acquire a hyperactivated motility,[29] the ability to bind to the zona pellucida and to undergo the acrosome reaction.[30]

The proper lipid composition of gametes is thus essential for fertilization, and transgenic mouse models have shown that dysregulation of cholesterol metabolism may act at different levels of male fertility.

CHOLESTEROL AND INFERTILITY – THE CONTRIBUTION OF TRANSGENIC MOUSE MODELS

Transgenic mouse models have been used to investigate the physiological importance of cholesterol homeostasis regulators (**Table 1**). In some models, male fertility studies have only been performed for regarding testicular disturbances (Retinoid X Receptor, *Rxr*$^{-/-}$ mice[31] – ATP-binding cassette, *Abca1*$^{-/-}$ mice[32]) and will not be developed here. In other cases, an epididymal phenotype was revealed but not in direct relation to cholesterol homeostasis. The *ApoER2*$^{-/-}$ (Apolipoprotein E receptor 2) mice, for example, suffered from infertility, but no alterations were detected in testicular or epididymal structures and sperm production was normal.[33] Even though ApoER2 is a member of the LDL receptor family, highly expressed in the principal cells of epididymal proximal segment,[34] no clear phenotype related to cholesterol homeostasis was described, the infertility being due to functional defects of the sperm mitochondria and cell volume dysregulation.

In other models, the authors concluded that the infertility was due to testicular abnormalities while their results also strongly suggested posttesticular maturation disturbances. For example, male mice heterozygous for a null apolipoprotein B allele (*Apo B*$^{+/-}$), a structural component of several classes of lipoproteins (chylomicron, VLDL, IDL, LDL), have reduced fertility associated with a decreased cholesterol plasma level.[35] The urogenital tract appears normal, but the sperm count is mildly reduced, motility is decreased, and spermatozoa are unable to fertilize an oocyte in the presence of its zona pellucida.[36] The authors only evoked the hypothesis of an unknown role of ApoB in spermatogenesis. However, today the importance of epididymal maturation is well-demonstrated for the acquisition of mobility and zona pellucida recognition, and ApoB is also expressed in the epididymis.[36] Defects could thus come from posttesticular events. Since this work, the authors have not pursued their investigation on this model.

Table 1: Transgenic mice used for genes involved in cholesterol-related biological processes and their associated male fertility phenotypes

Gene name	*Gene product*	*Biological process related to cholesterol*	*Model/reference*	*Phenotype*
Abca1	ATP-binding cassette, subfamily A, member 1	Cholesterol efflux and reverse transport	*Abca1*$^{-/-}$ mice Selva *et al.*[32] 2004	Fertility is decreased by 21% Lipid accumulation in Sertoli cells No sperm abnormalities
ApoB	Apolipoprotein B	Cholesterol transport and positive regulation of storage	*ApoB*$^{+/-}$ mice Huang *et al.*[36] 1996	No abnormalities in the testis Decrease in sperm motility Unable to fertilize an oocyte with zona pellucida
Dicer1	Dicer1, ribonuclease type III	Maturation of pre-microRNA	*Dicer1*$^{-/-}$ mice Björkgren *et al.* 2012–2014	Increased sperm immotility and broken cells Increased cholesterol/PUFA ratio in sperm Alteration of lipid homeostasis in epididymis Decrease in ability to bind and fertilize an oocyte
Lrp8	Low-density lipoprotein receptor-related protein 8, Apolipoprotein E receptor	Receptor for Reelin and apoE	*Apoer2*$^{-/-}$ mice Andersen *et al.*[33] 2003	Abnormal sperm morphology and immotility Cell volume dysregulation Mitochondrial abnormalities Decrease of the PHGPx protein in sperm
Npc1	Niemann-Pick type C1	Cholesterol efflux	*Npc1*$^{-/-}$ mice Fan *et al.*[38] 2006	Decreased sperm count Abnormal sperm morphology Unable to fertilize an oocyte with zona pellucida Defect in epididymal maturation of some proteins of the ADAM family
Npc2	Niemann-Pick type C2	Abundant component of the epididymal fluid Cholesterol binding activity *in vitro*	*Npc2*$^{-/-}$ mice Busso *et al.*[54] 2014	Decreased sperm cholesterol content Defect in capacitation Unable to fertilize an oocyte with zona pellucida
***Nr1 h2* (LXRα)** ***Nr1 h3* (LXRβ)**	Nuclear receptor subfamily 1, group H, members 2 and 3	Negative regulation of cholesterol storage Positive regulation of cholesterol efflux and transport	*Lxrα;β*$^{-/-}$ mice with high cholesterol diet Ouvrier *et al.*[49] 2011	Decreased sperm motility and viability Increased premature acrosome reaction and sperm abnormalities Alteration of lipid homeostasis in epididymis Trans-differentiation of smooth muscle cells into foam cells
Rxrb	Retinoid X receptor β	Transcription factor	*Rxrβ*$^{-/-}$ mice Kastner *et al.*[31] 1996	Oligo-astheno-teratozoospermia Lipid accumulation in Sertoli cells Degeneration of the seminiferous tubules

Inactivation of these genes in mouse models results in reduced fertility or infertility. Phenotypes indicated in bold are due to an alteration of the posttesticular maturation.
PUFA: polyunsaturated fatty acid; PHGPx: phospholipid hydroperoxide glutathione peroxidase

The Niemann-Pick C1 (NPC1) protein regulates the trafficking of low-density lipoprotein-mediated endocytosed cholesterol.[37] *Npc1*$^{-/-}$ male mice are infertile mostly because of a partial arrest of spermatogenesis.[38] Furthermore, spermatozoa show morphological head abnormalities and are unable to fertilize an oocyte *in vitro*.[38] These gametes also have a fault in a disintegrin and metalloprotease (ADAM) family protein maturation, in particular ADAM 3 (cyritestin), known to be essential for fertilization.[39] This protein must be cleaved during epididymal transit to be functional[40] and sperm cells of *Npc1*$^{-/-}$ mice show a defect in this cleavage, demonstrating a lack of epididymal maturation, however the authors did not discuss this point.

In these models, the posttesticular sperm defects were often not considered, even though several studies have demonstrated the deleterious impact of dyslipidemia on the posttesticular maturation of gametes and therefore fertility.

The impact of hypercholesterolemia on gamete epididymal maturation has regained some interest since our group has developed a model of diet-induced posttesticular infertility. This model combines knock-out mice for Liver X Receptors (LXRs) with a high cholesterol diet. The transcription factors LXRα (LXRα – *Nr1h3*) and LXRβ (LXRβ – *Nr1h2*) are activated by oxidized forms of cholesterol and have been shown to control the expression of genes involved in cholesterol homeostasis, differentiation, proliferation, inflammation, and reproduction.[41–45] Male mice deficient for the two LXR isoforms become totally infertile at 8–9 months, showing a composite phenotype: testicular[44] and epididymal (restricted to the proximal caput epididymis[46–48]). Young LXR-deficient male mice (3–4 months) are fertile and do not show any phenotype at all.[44,46] However, when young males are fed for 1 month with a high cholesterol diet (HCD, which provokes a large elevation of plasma LDL cholesterol or LDL-C), infertility is triggered and only the epididymal phenotype appears, showing the sensitivity of this organ to a cholesterol overload.[49] These animals recapitulate the epididymal phenotype of old mice: accumulation of cholesteryl esters in the proximal caput epididymidis, and disruption of the proximal epididymal segments (S1 and S2) with a reduction in the height of the epithelium. These mice present an "epididymosclerotic" phenotype characterized by a global loss of smooth muscle cells accompanied by trans-differentiation of these cells into "foam cells". These cells appeared to migrate towards and infiltrate the epididymal epithelium, which was corroborated by the presence of the matrix metalloproteinase MMP9. Testes showed no alteration, but the gametes were strongly affected. Motility and viability were decreased, and broken sperm cells were increased, showing a weakness of the head/flagellum junction. Premature acrosomal reactions were also increased and could result from an alteration in the membrane composition. This work clearly showed that, in this model, the epididymis is an early target of lipid-induced infertility. Given the importance of gamete lipid maturation during epididymal transit, we strongly suspect that infertility in this model is caused by an alteration in the membrane lipid composition of spermatozoa. This lipid composition will be crucial especially during capacitation, and this aspect is currently being studied.

Cholesterol homeostasis involves different proteins regulating intracellular trafficking. Niemann-Pick C2 (NPC2) is a lysosomal, soluble protein shown to be a cholesterol-binding protein relevant for the efflux of cholesterol from this organelle.[50] This protein has been shown to be one of the most abundant proteins in human epididymal fluid[51] and seems to participate in cholesterol efflux from spermatozoa during epididymal maturation.[52,53] NPC2 deficient mice (*Npc2*$^{-/-}$), a model for Niemann-Pick disease type C2 (neurological disease), are also infertile because they do not mate (locomotor dysfunction). However, given the abundance of NPC2 in the epididymis, it was of interest to check whether the spermatozoa of these males were able to fertilize an oocyte. The authors showed that *IVF* rates were significantly lower with spermatozoa from these mice than with those from the wild type.[54] Spermatozoa appeared normal in count, morphology, viability and mobility. After vasectomy and even after a vaso-vasostomy, NPC2 expression in the human epididymis is selectively down-regulated, and the sperm cholesterol content is increased,[53] confirming the functional role of NPC2 in cholesterol efflux. In bulls, purified NPC2 is able to reduce the cholesterol content of spermatozoa and to dissociate a large part of P25b molecules from lipid rafts, suggesting that NPC2 is involved in sperm membrane organization.[52] Unexpectedly, in the mouse, the lack of NPC2 appears to cause a decrease in sperm plasma cholesterol. Spermatozoa of *Npc2*$^{-/-}$ animals show reduced capacitation-induced cholesterol efflux, highlighted by a defect in tyrosine phosphorylation. The lower cholesterol content, with increasing membrane fluidity, may alter the distribution of molecules in noncapacitated spermatozoa. Indeed, as mentioned previously, lipid rafts (enriched in cholesterol) allow regionalized acquisition of proteins.[21] An impairment of this process, by a reduction in membrane cholesterol content, could ultimately affect the formation of capacitation-related signaling complexes during incubation under capacitation conditions.[54] Changes in sperm cholesterol content, either an increase or a decrease in these animals, have a direct impact on capacitation and thus on the ability to fertilize an oocyte.

More recently, a specific model of invalidation of Dicer1 in the caput epididymidis was generated (Dicer 1 cKO[55]) by using a mouse line expressing *iCre* under the Defensin beta 41 (*Defb41*) promoter.[56] Dicer1 is the RNAse III enzyme required for processing pre-microRNAs into their mature forms. These male mice were infertile, and the epithelial layer of both initial segment (IS) and caput epididymidis was disorganized. With age, the Dicer1 cKO IS morphologically resembled that of an undifferentiated prepubertal epididymis. Moreover, the authors showed a decrease in segment-specific gene expression and altered sex steroid receptor ratio.[55] Further examination of these Dicer1 cKO mice sperm showed that 33% of sperm cells were broken and that there was an increased spontaneous loss of the acrosome. Eventually, 80% of the spermatozoa were immotile. Such defects could be caused by membrane instability coming from the abnormal lipid composition of these gametes. The authors showed that under capacitating conditions Dicer1 cKO mouse spermatozoa did not lose cholesterol as did wild-type spermatozoa, leading to ineffective capacitation (only a small increase in tyrosine phosphorylation levels). Furthermore, the polyunsaturated fatty acid (PUFA) level was decreased compared with the wild type, consequently increasing the cholesterol/PUFA ratio and, therefore, the membrane rigidity. In the epididymis, expression levels of genes involved in the biosynthesis (*Hmgcr*, *Hsd17b7,* and *Dhcr24*) and transport (*Abca1* and *Abcg1*) of cholesterol were up-regulated.[57] This is in accordance with the lack of DICER1, thus strongly reducing the presence of all mature miRNAs from the cells, and ultimately giving rise to up-regulation of target genes. MicroRNAs have previously been shown to be involved in the regulation of lipid metabolism: miR-33 inhibits the expression of *Abca1* and *Abcg1*[58] in mice and miR-122 inhibits *Hmgcr*.[59] In the Dicer1 cKO, these miRNAs may not be matured, thus explaining the increase in their target gene expression levels, but this hypothesis will need confirmation. This original KO has brought new evidence regarding the importance of cholesterol homeostasis and miRNAs in the epididymis for proper maturation of spermatozoa.

MODELS OF DIET-INDUCED MALE INFERTILITY

Besides transgenic mouse models, hypercholesterolemia can also be diet-induced, mimicking nutritional dyslipidemia. The rabbit is a useful model because hypercholesterolemia can be easily triggered with cholesterol- or fat-enriched diets.[60,61] The observed diet effects vary depending on diet composition and duration. The main sperm modifications triggered by hypercholesterolemia are (1) an increase in the cholesterol content and the percentage of morphologically abnormal spermatozoa, (2) a decrease in sperm motility and the progesterone-induced acrosome reaction and (3) a constant sperm count.[62,63] The observed higher cholesterol content is an indicator of decreased membrane fluidity, a fact in accordance with an inhibition of capacitation shown by a reduced ability of spermatozoa to reach normal tyrosine phosphorylation levels. The link was established using a cell-permeant synthetic cAMP analog and phosphodiesterase inhibitors, which artificially increase intracellular cAMP levels and lead to a direct activation of PKA, bypassing the early membrane events of capacitation. Under these conditions, phosphotyrosine profiles are restored showing that the capacitation defect is associated with membrane-related events.[63]

The rat model has been less used for the reason that rats can physiologically manage a cholesterol overload.[61] Several studies with high cholesterol[64,65] or high fat-fed rats show quite similar results to what has been described in the rabbit:[66] decreased sperm motility, viability and sometimes sperm numbers,[64,65] and usually the percentage of morphologically abnormal sperm cells is increased.[65,66] In all these cases, testicular histology was disrupted, with a decrease in the seminiferous tubule diameter,[64] testicular atrophy[65] or a decrease in the number of Leydig and Sertoli cells.[66] These models are thus not appropriate to study the impact of hypercholesterolemia on epididymal sperm maturation.

CLINICAL DATA LINKING CHOLESTEROL PLASMA LEVELS AND POSTTESTICULAR MATURATION EVENTS

The first report linking modification of cholesterol metabolism and semen parameters in the human was published in 1993 by Dobs and colleagues. A statistically significant decrease in sperm motility was evident in a subset of 14 male patients included in a Randomized Control Trial (RCT) assessing the efficiency of pravastatin compared with cholestyramine.[67] Interestingly, these LDL-lowering drugs act through independent mechanisms. Pravastatin belongs to the statin family of lipid-altering agents. They are competitive inhibitors of Hydroxymethylglutaryl Coenzyme A (HMG-CoA) reductase (HMGR), the rate-limiting enzyme in cholesterol biosynthesis, which irreversibly reduces 3-Hydroxy-3-methylglutaryl Coenzyme A (HMG-CoA) to mevalonate. By occupying a portion of the binding site of HMG-CoA to the enzyme, they block the access of the substrate to the catalytic site of HMGR, which results in a decrease in intracellular cholesterol. The total cholesterol- and low-density lipoprotein-cholesterol (LDL-C)-lowering effect of statins is then explained by their ability to decrease cholesterol production in the liver. [68]

Cholestyramine belongs to the family of Bile Acid Sequestrants (BAS). They consist of large polymers that are able to bind to bile salts in the intestine, preventing their reabsorption from the gut and increasing their fecal excretion. Increased fecal excretion leads to a subsequent shift in bile salt *de novo* synthesis from liver cholesterol at the expense of LDL-C synthesis, which results in a lowering of blood LDL-C levels.[69] In the initial Dobs *et al.* study, a decrease in sperm motility was found 6 and 12 months after treatment indicating that this adverse event was the result of a lowering of total cholesterol and LDL-C, and not a specific side effect of a particular treatment. This finding could also not be linked to an alteration of steroid metabolism suggesting that LDL-cholesterol lowering could be acting on posttesticular events, such as epididymal sperm maturation, as epididymides are involved in the acquisition of sperm motility. The authors also noticed a significant decrease in semen volume and sperm count at 6 months, but it returned to baseline after 12 months. This may reflect a transient decrease in testosterone levels since testosterone is essential for normal spermatogenesis and the function of the male accessory glands (epididymides, prostate, and seminal vesicles). However, that testosterone levels and testosterone responses to hCG were not significantly affected during the study, which ruled out this hypothesis.

Interestingly, one blind trial evaluating the impact on testicular function of simvastatin, another molecule of the statin family, found no differences in sperm count or motility after 14 weeks of treatment.[70]

The long-term effect of pravastatin was further evaluated in eight hypercholesterolemic patients, and no significant changes in values of semen parameters, including motility, were found after 6 months of effective treatment.[71] Another RCT comparing the effect of two statins, pravastatin (n = 39), simvastatin (n = 41) and placebo (n = 39) on testicular function and androgen production in hypercholesterolemic patients was published by the same author who first identified a decrease in sperm motility after lowering of LDL-cholesterol.[72] This time, no significant changes in semen quality were found after 12 and 24 weeks of treatment and testicular steroid secretion was not altered.

Very recently, a prospective noncontrolled pilot assay aimed at evaluating the impact of a 5-month therapy with atorvastatin on semen parameters of 17 healthy, normo-cholesterolaemic and normozoospermic patients.[73] Treatment was significantly associated with a decrease in sperm number, sperm vitality, and seminal concentration of epididymal (neutral alpha 1–4 glucosidase, L-carnitine) and prostatic (acid phosphatase) markers. Sperm morphology was significantly altered in the head, neck, and midpiece regions, and the Multiple Anomalies Index was moderately increased. The percentage of acrosome-reacted spermatozoa was significantly decreased after treatment, but the cholesterol and phospholipid content and ratio in spermatozoa and seminal plasma were unchanged. Surprisingly, sperm motility was significantly but slightly increased at the end of the 5-month treatment. The authors concluded that atorvastatin treatment impaired the fertility potential of the volunteers at the testicular, epididymal and prostatic levels, and that this effect was independent of an alteration of the hypothalamic pituitary testicular axis, as plasma gonadotropin and testosterone levels remained stable during the study. Hypotheses were made on a direct effect of atorvastatin on gonadal, epididymal, and prostatic functions, as some factors identified as determinants of male fertility are classically decreased by statin molecules. This is the case for coenzyme Q10, a provitamin with antioxidant properties synthesized by HMGR. However, a direct effect of total cholesterol- and LDL-C- lowering on epididymides could also be responsible for some of the findings described in the study, especially as total cholesterol and LDL-C were normal before treatment onset. Even though to our knowledge, there are no links between hypocholesterolemia and male infertility in the current scientific literature, this aspect should not be ignored and future studies evaluating the effect of statins on male fertility should include a control group treated with unrelated lipid-lowering drugs.

Interestingly, the same team have previously linked hypercholesterolemia with an increase in seminal carnitine, a biomarker of epididymal function implicated in sperm maturation, metabolism, and motility.[74] Neither changes in sperm or seminal plasma lipid content nor an alteration of other male accessory gland

function (prostate and seminal vesicle markers) or testosterone levels were found, suggesting that hypercholesterolemia acted solely on carnitine production by the epididymides. Interestingly, there was also no significant difference in basic semen parameter values between hypercholesterolemic ($n = 11$) and control men ($n = 11$), although the number of subjects was too low to be clinically relevant.

Taken together, these results suggest that hypercholesterolemia may act on human epididymides by increasing L-carnitine secretion, and that total cholesterol- and LDL-C- lowering could impede sperm motility in previously hypercholesterolemic patients, although available studies have shown contradictory results for the latter finding.

In previously normocholesterolemic and normozoospermic volunteers, total cholesterol- and LDL-C- lowering after 5 months of atorvastatin treatment were associated with a decrease in sperm count, epididymal and prostatic seminal markers, combined with an increase in sperm motility. Even if atorvastatin toxicity through direct or off-target effects could explain these results, a direct action of cholesterol-lowering on epididymides should not be ruled out.

INSIGHTS ON HUMAN FERTILITY OBTAINED FROM TRANSGENIC ANIMAL MODELS

As stated above, in a murine KO model, LDL-C can act on epididymal function through its interaction with ApoER2, a member of the LDL receptor family acting both in endocytosis and in signal transduction through the SRC kinase and Phosphatidyl Inositol-3 kinase pathways. ApoER2 null mice are infertile and present with an alteration of sperm motility and morphology.[33] Abnormal morphology consists of tail coiling, bending, angulation, and midpiece deformation involving axonemal structures. These impairments are the results of posttesticular maturation events taking place in the initial portion of the mouse epididymis and involve a decrease in Phospholipid Hydroperoxide Glutathione Peroxidase (PHGPX) protein levels. PHGPH (or GPx4) is a selenoprotein belonging to the family of glutathione peroxidases. In spermatozoa, PHGPX is located in the midpiece and catalyzes the reduction of lipid peroxides, conferring a protective effect against ROS generation by the mitochondrial respiratory chain and subsequent sperm lipid peroxidation. Interestingly, a decrease in sperm PHGPX expression has been found in human males presenting with idiopathic asthenozoospermia.[75–77] It has also been implicated in the postthaw survival of human spermatozoa.[78] However, the alteration of PHGPX expression could not be linked to mutations in the gene, or with single nucleotide polymorphisms of the 5'-untranslated region of the related mRNA,[79] and so the mechanism linking ApoE2R and PHGPX expression still remains unclear.

As described above, hypocholesterolemic ApoB$^{+/-}$ mice are infertile with a decrease in sperm count, motility and zona binding, suggesting an alteration of epididymal maturation. The phenotype is partially reversed by using human ApoB as a transgene. Genetic association studies have been subsequently performed in humans. Initial work reported a significant association between a 3-codon deletion polymorphism in the ApoB signal peptide gene and infertility in Slovene Caucasian patients presenting with oligo-astheno-teratozoospermia.[80] However, no correlation was found between the polymorphisms and basic semen parameter values, and such an association was not significant when a population of non-Caucasian Indian males was studied for the same deletion.[81]

In the murine model, Dicer 1 has been implicated in initial segment and caput epididymidis homeostasis, and associated with a dramatic decrease in sperm motility, capacitation, and an abnormal phospholipid/cholesterol ratio causing sperm membrane rigidity. This finding could be explained by the absence of mature miRNAs controlling the translation of genes implicated in cholesterol metabolism, such as *Abca1*, *Abcg1,* and *Hmgcr*, as reviewed above. In humans, polymorphism in key enzymes of miRNA pathways, Dicer1 and Drosha, have been associated with semen quality in a Chinese population. But after Bonferroni correction, the association only persisted with oligozoospermia for a polymorphism in Dicer1,[82] which does not favor an alteration of posttesticular maturation events. Specific miRNAs profiles have also been described in distinct segments of human epididymides[83] and in epididymosomes,[84] suggesting an important role of epididymal miRNAs in sperm maturation, as well as a possible uptake of these miRNAs by spermatozoa during epididymal transit. Very recently, it has been demonstrated that a sperm protein, cysteine-rich secretory protein 2 (CRISP2), involved in modulation of sperm motility, acrosome reaction, and gamete fusion, is regulated by miR-27b, a miRNA that has been described as one of 18 seminal plasma miRNAs specifically present in epididymosomes[84] and which is differentially expressed in the human epididymal corpus.[83] Indeed, a decrease of CRISP2 protein level in spermatozoa from asthenozoospermic patients has been linked to an increase of miR-27b. Furthermore, in a retrospective study, high miR-27b expression or low CRISP2 protein expression was significantly associated with low sperm progressive motility, abnormal morphology, and infertility.[85] MiR-27b was earlier described as a regulator of adipocyte differentiation, as expression of miR-27 resulted in blockade of expression of PPARγ and C/EBPα, the two master regulators of adipogenesis[86] and thus could represent a link between lipid metabolism, sperm epididymal maturation, and male infertility in humans.

CONCLUSION

Human clinical data linking hypercholesterolemia and epididymal function are scarce, and often based on studies evaluating the safety of cholesterol-lowering drugs, as cholesterol is a key player in steroid metabolism. However, some studies showed an association between alterations of total cholesterol and LDL-C levels, and markers of epididymal function, but no modifications of sperm membrane lipid content or capacitation were observed. Furthermore, transgenic animal models have identified several possible molecular targets involved in cholesterol metabolism that are implicated in alteration of posttesticular maturation events. Some, but not all of these molecular targets have already been studied in humans, such as PHGPX, NPC2, and ApoB. The effect of hypercholesterolemia on posttesticular maturation has also been described in animals fed with high-fat diets. The inability to reproduce these results in hypercholesterolemic humans could be due to the impossibility of reproducing such a diet, to the low number of subjects included in these studies or to a lesser sensitivity of the human than mouse epididymis to high cholesterol content. Thus, large comparative and prospective studies assessing basic and extended semen parameter values, as well as capacitation, fertilization biomarkers, and IVF data in hypercholesterolemic compared with control patients are needed to comprehend better the effect of hypercholesterolemia on human male fertility and the implications for future medical intervention.

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