High-density lipoprotein metabolism and the human embryo

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Background: High-density lipoprotein (HDL) appears to be the dominant lipoprotein particle in human follicular fluid (FF). The reported anti-atherogenic properties of HDL have been attributed in part to reverse cholesterol transport. The discoveries of the scavenger receptor class B type I (SR-BI) and the ATP-binding cassette A1 lipid (ABCA1) transporter have generated studies aimed at unraveling the pathways of HDL biogenesis, remodeling and catabolism. The production of SR-BI and ABCA1 knockout mice as well as other lipoprotein metabolism-associated mutants has resulted in reduced or absent fertility, leading us to postulate the existence of a human hepatic-ovarian HDL-associated axis of fertility. Here, we review an evolving literature on the role of HDL metabolism on mammalian fertility and oocyte development.

Methods: An extensive online search was conducted of published articles relevant to the section topics discussed. All relevant English language articles contained in Pubmed/Medline, with no specific time frame for publication, were considered for this narrative review. Cardiovascular literature was highly cited due to the wealth of relevant knowledge on HDL metabolism, and the dearth thereof in the reproductive field.

Results: Various vertebrate models demonstrate a role for HDL in embryo development and fertility. In our clinical studies, FF levels of HDL cholesterol and apolipoprotein A1 levels were negatively associated with embryo fragmentation, but not with embryo cell cleavage rate. However, the HDL component, paraoxonase 1 arylesterase activity, was positively associated with embryo cell cleavage rate.
CONCLUSIONS: HDL contributes to intra-follicular cholesterol homeostasis which appears to be important for successful oocyte and embryo development.

Key words: Embryo quality / Oocyte quality / Female infertility / Follicular fluid / Embryo development

Introduction

High-density lipoprotein (HDL) appears to be the dominant lipoprotein particle in follicular fluid (FF) during human folliculogenesis. The physiological role of HDL particles in the developing follicle has remained obscure, although it is well established that both HDL and low-density lipoprotein (LDL) deliver cholesterol to the corpus luteum as a substrate for progesterone synthesis (Roy and Belanger, 1992; Azhar et al., 1998a, b; Reaven et al., 1998). HDL plays an important role in cholesterol and phospholipid metabolism, providing for the dual exchange of lipids to and from cells within the human body. It is also well established that HDL particles can moderate reactive free radicals, thereby limiting oxidative damage to cells and other lipoproteins (Parthasarathy et al., 1990; Kunitake et al., 1992; Klimov et al., 1993; Decossin et al., 1995; Mackness and Durrington, 1995). Numerous animal models illustrating the effects of HDL metabolism on reproductive outcomes suggest that HDL cholesterol metabolism may be important in human reproduction (Nimpf et al., 1989; Bujo et al., 1994, 1995a, b; Trigatti et al., 1999; Christiansen-Weber et al., 2000; Miettinen et al., 2001; Aiello et al., 2003; Steffensen and Gustafsson, 2004; Yesilaltay et al., 2006).

In the course of our studies, we examined the role of HDL particles on assisted reproductive technology laboratory outcomes. Prior to the discussion that follows, it is critical to emphasize that the biosynthesis, remodeling and catabolism of HDL are complex and beyond the scope of this review. These processes are thoroughly presented elsewhere (Lewis, 2006; Rader, 2006; Zannis et al., 2006). The aim of this narrative review is to provide appropriate background and current knowledge relevant to HDL particles and human reproduction, generating specific testable hypotheses for future investigations addressing the role of HDL in reproduction.

Methods

This narrative review comprised an extensive online search of English publications listed in PubMed/Medline, which was first conducted on 31 January 2007 and was continuously updated through 14 January 2009. The initial combinations of subject headings employed to conduct the search were: embryo quality, mammalian OR oocytes OR infertility, female OR cleavage stage, ovum OR embryo fragmentation OR granulosa cells OR ovarian follicle OR follicular fluid OR steroidogenesis AND lipoproteins OR HDL OR scavenger receptor class B type 1 (SR-BI) OR HDL cholesterol. No limits were imposed by date of publication; however, articles with content judged to be of key importance in providing background knowledge fundamental to the aim of this review were identified by the lead author (V.Y.F.). As only 6 of 49 citations (Perret et al., 1985; Berger et al., 1987; Suchanek et al., 1988; Volpe et al., 1991; Miettinen et al., 2001; Browne et al., 2008) with direct relevance to HDL and embryo quality were identified, an additional search was broadly conducted of PubMed/Medline citations employing the paired terms oocyte development AND reverse cholesterol transport OR cholesterol metabolism OR lipoprotein metabolism OR HDL OR SR-BI OR ApoAI OR ABCA1 OR ABCG1 OR CETP OR LCAT through 16 January 2009. This secondary search provided 53 citations which were further evaluated for relevance and selected for inclusion in this review. It is notable that cardiovascular literature was highly cited due to the limited understanding about the role of lipoproteins in reproduction beyond steroidogenesis. All cross-references in relevant cardiovascular and reproductive journals were searched electronically. There were 172 citations identified employing this approach. However, the latter scenario speaks to the knowledge gaps present with regard to the role played by HDL metabolism in human reproduction.

HDL particle and its components

Our understanding of the HDL particle has evolved principally via cardiovascular literature. The anti-atherogenic and anti-inflammatory properties of HDL have been attributed to its role in reverse cholesterol transport and ability to restrict the oxidation of LDL (Mackness et al., 1991; Curtiss et al., 2006; McPherson et al., 2007; Vedhachalam et al., 2007). With a density range of 1.063–1.210 g/ml, HDL is smaller and denser than its counterpart particles, LDL and very low-density lipoprotein (VLDL).

Apolipoprotein Al (ApoAI) is the principal apolipoprotein associated with HDL and closely correlates with total HDL cholesterol levels (Santos-Gallego et al., 2008). Depending on subclass, ApoAI comprises 40–70% of the particle mass. Cholesterol and its esters comprise ~15% and phospholipids ~25% of the total mass of HDL particles (Havel and Kane, 1995). ApoAI is synthesized in the liver and intestine (Zannis et al., 1985) and plays a critical role in each stage of HDL biogenesis, remodeling and catabolism. HDL particle size and structure are influenced by phospholipid and cholesterol constituents, which alter the conformation of ApoAI (Davidson et al., 1995; Tricerri et al., 1998). Familial ApoAI deficiency results in undetectable ApoAI and markedly reduced HDL cholesterol levels (Schaefer et al., 1985; Bekaert et al., 1991; Santos et al., 2008). ApoAI-deficient mice also show a marked reduction in HDL levels (Williamson et al., 1992). Naturally-occurring ApoAI mutations reduce the ability of ApoAI to activate lecithin:cholesterol acyltransferase (LCAT), resulting in low HDL levels (Sorci-Thomas and Thomas, 2002). ApoAI polymorphisms have been associated with different HDL subclass distributions (Jia et al., 2005). In addition to ApoAI, the HDL particle may also include constituent proteins (ApoAI, ApoAIV, ApoAV, ApoE, ApoM, ApoC-I and ApoC-III), LCAT through 16 January 2009. This secondary search provided 53 citations which were further evaluated for relevance and selected for inclusion in this review. It is notable that cardiovascular literature was highly cited due to the limited understanding about the role of lipoproteins in reproduction beyond steroidogenesis. All cross-references in relevant cardiovascular and reproductive journals were searched electronically. There were 172 citations identified employing this approach. However, the latter scenario speaks to the knowledge gaps present with regard to the role played by HDL metabolism in human reproduction.
HDL particles have been described to consist of microemulsion particles stabilized by a phosphatidylcholine monolayer with protein at the aqueous interface. It is theorized that ApoAI exists as antiparallel, \(\alpha\)-helical dimers, forming ‘belts’ that extend around the periphery of spherical HDL particles (Koppaka et al., 1999; Panagotopulos et al., 2001; Davidson and Silva, 2005; Silva et al., 2005, 2008; Mishra et al., 2006). Mature HDL particles in blood range in size between 7 and 12 nm in diameter and in mass between 200 and 400 kDa. Thus, HDL particles are dynamically constructed with varying lipid content with small HDL particles having the least amount of core lipid (Segrest et al., 2000a, b).

No standard classification system for HDL particles exists. Nomenclature for HDL particles varies depending on the method of analysis. Initial attempts to characterize HDL into subclasses, based on gel electrophoresis migration patterns, resulted in the designations of \(\alpha\), pre-\(\beta\) and \(\gamma\) (Kunitake and Kane, 1982; Kunitake et al., 1985).

Large lipid-rich spherical HDL particles, designated as \(\alpha\)-migrating, comprise 85–95% of measurable plasma HDL cholesterol, whereas small lipid-poor HDL, or pre-\(\beta\) migrating, comprise the remaining 5–15% (Ishida et al., 1987; Assmann and Gotto, 2004). The \(\alpha\)-HDL particles can be further distinguished as two major subfractions: larger HDL2 and smaller HDL3. Plasma HDL gradient gel electrophoresis reveals five HDL subclasses: HDL2a (7.2–7.8 nm), HDL2b (7.8–8.2 nm), HDL3a (8.2–8.8 nm), HDL3b (8.8–9.7 nm) and HDL2b (9.7–12 nm) (Williams et al., 1997; Jia et al., 2006). Using nuclear magnetic resonance (NMR), five different subclasses of HDL particles have been characterized by size, with a designated number classification reflecting increasing diameter (H1, H2, H3, H4 and H5) (Kwitterovich, 2000; Santos-Gallego et al., 2008). However, current NMR clinical analyses generally report three HDL subclass designations: HDL large particles, HDL medium particles and HDL small particles (Otvos et al., 2006).

**HDL biogenesis, remodeling and catabolism**

The current model of HDL biogenesis is one in which the ATP-binding cassette A1 (ABCA1) transporter on the surface of liver and intestinal epithelial cells participates in the initial lipidation of lipid-free ApoAI via the transfer of cellular phospholipids and cholesterol (Fig. 1) (Wang et al., 2000; Remaley et al., 2001; Brunham et al., 2006). Unesterified cholesterol is also transferred from macrophages by the ATP-binding cassette G1 (ABCG1) transporter (Terasaka et al., 2007). After formation of these nascent lipid-poor HDLs, lipidated ApoAI is transformed into lipid-rich HDL. In the presence of other plasma lipoproteins, additional cholesterol and lipids are transferred to and from HDL by the lipid transfer proteins, cholesterol ester transfer protein (CETP) and phospholipid transfer protein (PLTP). CETP transfers core HDL CEs to other lipoprotein particles (Rye and Barter, 2004), whereas PLTP promotes phospholipid exchange between HDL and triglyceride-rich LDL and VLDL (Jauhiainen et al., 1993; Tu et al., 1993; Lusa et al., 1996; von Eckardstein et al., 1996; Jiang et al., 1999). Further catabolic remodeling of HDL is mediated by several lipases including lipoprotein lipase, hepatic lipase and endothelial lipase (Guendouzi et al., 1998). This lipid remodeling involving bidirectional lipid exchange with cells and other lipoproteins is referred to as ‘HDL maturation’ (Martinez et al., 2004).

HDL maturation generates spherical HDLs which interact with the cellular murine SR-BI to deliver CEs intracellularly to liver and steriodogenic tissues via a process called ‘selective lipid uptake’, yielding small, lipid-poor HDLs that are again suited for cholesterol efflux (Fig. 1) (Glass et al., 1983; Stein et al., 1983; Reaven et al., 1984, 1995; Acton et al., 1994; Acton et al., 1996; Rigotti et al., 1997a, b). Murine SR-BI can also participate in the reverse cholesterol transport of intracellular cholesterol to the growing HDL particle (Ji et al., 1997; Gu et al., 2000; Yancey et al., 2000; Ohgami et al., 2001). Murine SR-BI is a multiligand receptor that directly binds to apolipoproteins to mediate lipid transfer into cells (Xu et al., 1997; Calvo et al., 1998; Liadaki et al., 2000). Murine SR-BI is a 509 amino acid membrane protein with a large extracellular loop belonging to the CD36 superfamily of proteins (Acton et al., 1994; Krieger, 1999). SR-BI receptors exist in cholesterol efflux-mediating microdomains called caveolae in the plasma membrane (Fielding and Fielding, 1995; Babitt et al., 1997; Graf et al., 1999a, b). Caveolin-1 is an important cholesterol trafficking protein in caveolae that has been implicated...
to have a role with SR-BI-mediated cholesterol efflux (Graf et al., 1999a, b; Matveev et al., 1999; Frank et al., 2002; Wang et al., 2003). A PDZK1-interacting domain has been identified on SR-BI that induces cell surface expression of SR-BI in the liver, suggesting an important regulatory role for PDZ domain proteins (Ikemoto et al., 2000; Kocher et al., 2003).

CLA-1 receptor (CD36 and LIMPII analogous 1), the 509 amino acid human homolog of rodent SR-BI, also interacts with LDL to promote both selective lipid uptake and reverse cholesterol transport (Calvo and Vega, 1993; Calvo et al., 1995; Gao et al., 1997; Liu et al., 1997; Murao et al., 1997). The mechanism of selective lipid uptake into cells by SR-BI/CLA-1 receptors is uniquely different from the lipid uptake process involving LDL receptors. LDL receptor lipid uptake generally occurs via receptor-mediated endocytosis of the intact LDL particle, whereas SR-BI interacts with lipoproteins to effect selective lipid exchange (Goldstein et al., 1985; Acton et al., 1996; Krieger, 1999). This selective lipid exchange with SR-BI appears to favor lower density, larger, higher lipid content α-HDL particles, whereas pre-β-HDL and lipid-free ApoAI are poor substrates for SR-BI (Liakaki et al., 2000; de Beer et al., 2001). SR-BI and CLA-1 are highly regulated by cholesterol and steroidogenic pathway mediators including trophic hormones, and steroid hormones including estrogen, fatty acids and micronutrients (Krieger, 1999; Spady et al., 1999; Witt et al., 2000; Lopez et al., 2002).

Reverse cholesterol transport is an important mechanism for HDL particle remodeling. In addition to SR-BI, the ABCA1 transporter protein has been identified to promote cholesterol efflux albeit to lipid-poor ApoAI but not to spherical HDL particles (Zannis et al. 2006). Recent identification of novel proteins (ABCG1 and G4 transporter proteins) suggests that cholesterol efflux also occurs with intermediate-sized HDL particles, the immediate products of ABCA1-mediated ApoAI lipidation (Wang et al., 2004; Curtis et al., 2006). Circulating spherical HDL can generate cholesterol efflux competent particles through the action of PLTP which has been shown to have the ability to dissociate ApoAI from spherical HDL, converting HDL3 particles into two populations of large (HDL2) and small particles (pre-β-HDL) (Jauhiainen et al., 1993; Tu et al., 1993; Pussinen et al., 1995; Lusa et al., 1996). Pre-β-HDL can then participate in ABCA1-mediated efflux while the HDL2 particle undergoes selective cholesterol uptake by the liver via SR-BI in mice and CLA-1 in humans (Brown et al., 1989; Inazu et al., 1990; Silver et al., 2001). Cholesterol is subsequently transported via hepatocytes into bile while the lipid-poor HDL particles are recycled to serve again in the reverse cholesterol transport process (Rigotti et al., 1997a, b; Varban et al., 1998). ABCA1 transporter and LCAT mutations have been described to result in HDL deficiencies (Cohen et al., 2004; Sviridov and Nestel, 2007). The essential role of cholesterol efflux in intracellular cholesterol homeostasis is demonstrated by the multiplicity of other exchangeable lipoproteins, such as ApoE, ApoAI, ApoCII, ApoCIV, ApoAIV and ApoAV, which are also involved in HDL cholesterol efflux (Remaley et al., 2001; Beckstead et al., 2003; Mahley et al., 2006). Although ApoAI is the principal apolipoprotein involved in cholesterol efflux, these other apolipoproteins with the exception of ApoAV appear to promote reverse cholesterol transport via the ABCA1 transporter (Gillotte et al., 1999; Remaley et al., 2001; Santamarina-Fojo et al., 2001; Beckstead et al., 2003).

In addition to the aforementioned lipases and lipid transport proteins, HDL also contains unique enzymes belonging to the paraoxonase family, PON1 and PON3, which are believed to protect cells, LDL and lipids from oxidative and peroxidative modifications (Mackness et al., 1991; Primo-Parmo et al., 1996; Reddy et al., 2001; von Eckardstein et al., 2005; Negre-Salvayre et al., 2006; Draganov, 2007). Both PON1 and PON3 are synthesized in the liver and transported in the circulation associated with HDL particles. PON1 protein expression (Angelucci et al., 2006), along with its paraoxonase and arylesterase activities, and PON3 activity have been detected in human FF (Browne et al., 2008).

To summarize, the complexity of HDL metabolism stems from the inter-relatedness of its many proteins, lipids and apolipoproteins. HDL constitution affects its conformation and component activities as well as binding to its receptors, SR-BI and ABC transporter proteins. The ability of HDL to effect selective lipid uptake and reverse lipid transport subsequently alters the composition and function of HDL particles. It is the dynamic modeling of HDL within FF with potential downstream effects on oocyte development and embryo quality which is the primary focus of this review.

### HDL particles and ovarian steroidogenesis

The role of SR-BI-mediated selective uptake in the acquisition of cholesterol for steroidogenesis has been well studied and a thorough review is beyond the scope of this manuscript (Azhar et al., 1998a, b; Azhar and Reaven, 2002). To summarize, our current understanding of cholesterol utilization for steroidogenesis is based on progestosterone biosynthesis occurring within the luteinized granulosa and thecal cells of the corpus luteum. HDL and LDL provide cholesterol for steroidogenesis via various mechanisms: (i) selective CE uptake; (ii) receptor-mediated endocytosis of cholesterol; (iii) intracellular de novo biosynthesis; (iv) utilization of plasma membrane cholesterol; and (v) utilization of stored intracellular lipid droplet CE (Glass et al., 1983; Goldstein et al., 1985; Azhar and Reaven, 2002). The SR-BI-mediated mechanism of selective CE uptake utilizing both HDL and LDL particles to provide a high-capacity cholesterol delivery system was initially demonstrated in the adrenal gland (Rigotti et al., 1996; Temel et al., 1997). It was similarly shown that this mechanism is utilized by the corpus luteum to produce large quantities of progesterone (Azhar et al., 1990, 1998a, b; Reaven et al., 1995, 1996). The selective CE uptake pathway of lipoprotein cholesterol delivery is stimulated by gonadotrophin hormones via a cyclic AMP mechanism (Azhar et al., 1998a, b, 1999; Reaven et al., 1999). Enzymes such as phospholipase A2, hepatic lipase and lipoprotein lipase appear to regulate the selective uptake pathway of HDL CE into steroidogenic cells (Komaromy et al., 1996; Bruggen et al., 1998; de Beer et al., 2000; Lambert et al., 2000; Seo et al., 2000; Merkel et al., 2002). Specialized cell surface structures on steroidogenic cells with microvilli-forming channels efficiently expose lipoproteins such as HDL to SR-BI receptors, thereby facilitating the selective uptake of CE for steroidogenesis (Reaven et al., 1988, 1989, 1990).
Evidence from lipoprotein metabolism-associated gene knockouts and mutations

The discoveries of the HDL receptor SR-BI (Acton et al., 1996) and the ABCA1 lipid transporter (Bodzioch et al., 1999; Brooks-Wilson et al., 1999; Langmann et al., 1999; Remaley et al., 1999; Rust et al., 1999) have provided critical links to understanding the metabolism of HDL particles. The gene knockout (KO) models for these proteins have provided additional important clues to the importance of HDL in reproduction. The reproductive deficiencies of SR-BI and ABCA1 transporter protein KO female mice are described in this section. It is important to note that the LCAT KO female mouse is fertile with elevated pre-β-HDL particles (Sakai et al., 1997). Despite the absence of ApoAI, ApoAI KO female mice also appear to be fertile, perhaps due to the multiplicity of apolipoproteins involved in HDL-mediated reverse cholesterol transport (Plump et al., 1997).

We have summarized the various strains of animals with gene mutations or KOs fundamental to lipoprotein metabolism which display abnormal lipid levels, abnormal lipoprotein particles (particularly HDL) and reduced or absent female fertility in Table I.

Murine SR-BI KO model

The establishment of a murine SR-BI KO model provided the opportunity to better understand the role of SR-BI in cholesterol homeostasis (Rigotti et al., 1997a, b). The most compelling evidence for the importance of HDL in mammalian oocyte development and competence is exemplified by studies of SR-BI KO mice (SR-BI−/−) (Trigatti et al., 1999; Miettinen et al., 2001; Yesilaltay et al., 2006). SR-BI plays a key role in the metabolism of lipoproteins by mediating the uptake of cholesteryl esters from HDL and other lipoproteins into cells via a mechanism called selective lipid uptake (Zannis et al., 2006).

In studies of SR-BI KO mice, plasma HDL particles display a distinctive abnormality characterized by abnormally large, ApoE-rich particles with a high unesterified cholesterol:total cholesterol ratio (UC:TC ratio) (Braun et al., 2003; Van Eck et al., 2003). Female SR-BI KO mice when bred with wild-type males were infertile secondary to the uniform arrest of embryos at the zygote or 2-cell stages (Trigatti et al., 1999; Rigotti et al., 2003). No gross morphological abnormalities in SR-BI KO ovaries were noted. The phenotype of the female SR-BI KO mouse included: (i) reduced CE storage in the ovaries, without decreased progesterone production; (ii) lack of defects in estrus cycles or number of oocytes ovulated; and (iii) abnormal morphology in unfertilized oocytes and embryos (Trigatti et al., 1999). Figure 2 is reproduced from Trigatti et al. (1999), demonstrating the abnormal non-refractive morphology and development of preimplantation embryos from SR-BI KO mice. Trigatti et al. (1999) also observed a similar non-refractive morphology in wild-type oocytes after exposure to cholesterol-binding chemicals, nystatin and filipin.

![Figure 2](image-url) In vivo ovarian lipid accumulation in and in vitro development of preimplantation embryos from wild-type and SR-BI KO mice. Six-week-old female mice were superovulated and were mated to males of the other genotype (i.e. SR-BI+/− females mated to SR-BI−/− males and vice versa) to generate embryos with heterozygous mutant genotypes. Ovaries and preimplantation embryos were harvested the next morning (Day 0). (A and B) Typical oil red O staining of lipids in ovaries from SR-BI+/+ (A) or SR-BI−/− (B) animals. The arrows indicate corpora lutea. (Bar = 450 μm.) (C and D) Phase-contrast microscopy of preimplantation embryos (cultured for 1 day) from SR-BI+/+ (C) or SR-BI−/− (D) females mated to males of the opposite genotype. Similar results were observed when SR-BI−/− males were mated to SR-BI+/− females. Open arrowheads indicate morphologically normal, 1- or 2-cell embryos; solid arrowheads indicate embryos with abnormal, non-refractive morphology. (Bar = 100 μm.) (E) Plasma progesterone concentrations from pseudo-pregnant females (6 days post-mating, ages 6–10 weeks, weight = 19–25 g, n = 8; P = 0.08). (F) Percentage of preimplantation embryos from SR-BI+/+ (open bars) or SR-BI−/− (solid bars) females with normal morphology during 3 days of culture. The values represent the averages from five animals of each genotype. Total number of embryos: SR-BI+/+, 131; SR-BI−/−, 167. Reprinted from Trigatti et al. (1999), permission granted from Proceedings of the National Academy of Sciences, Copyright 1999.

### Table I Summary of various mammalian gene-KO or avian mutant models associated with HDL metabolism and reproductive effects

<table>
<thead>
<tr>
<th>Model</th>
<th>Infertile</th>
<th>Reduced litter size</th>
<th>Phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>SR-BI−/−</td>
<td>Yes</td>
<td>—</td>
<td>Zygote, 2-cell embryo arrest</td>
</tr>
<tr>
<td>LCAT−/−</td>
<td>No</td>
<td>No</td>
<td>Fertile</td>
</tr>
<tr>
<td>R/O Mutant Chicken</td>
<td>Yes</td>
<td>—</td>
<td>Lack of yolk formation</td>
</tr>
<tr>
<td>ABCA1−/−</td>
<td>No</td>
<td>Yes</td>
<td>Embryopathy</td>
</tr>
<tr>
<td>LXR−/−</td>
<td>No</td>
<td>Yes</td>
<td>Oocyte nuclear maturation defect</td>
</tr>
<tr>
<td>ApoAI−/−</td>
<td>No</td>
<td>—</td>
<td>Fertile</td>
</tr>
</tbody>
</table>

HDL, high-density lipoprotein; SR-BI, scavenger receptor, class B type I; LCAT, lecithin–cholesterol acyltransferase; R/O, restricted ovulator; ABCA1, ATP-binding cassette A1 transporter; LXR, liver X receptor; ApoAI, apolipoprotein 1.
Miettinen et al. (2001) subsequently employed surgical, pharmacological and genetic interventions to study the infertility in SR-BI KO mice. Restoration of fertility in the SR-BI KO model occurred when (i) SR-BI−/− ovaries were transplanted into wild-type mice, indicating that the fertility impairment was not due to the SR-BI receptor-deficient ovary per se; (ii) probucol, a cholesterol-reducing agent (Zhang et al., 1997; Yamamoto, 2008), was administered to SR-BI KO mice, which lowered cholesterol levels and normalized the plasma HDL UC:TC ratio (Braun et al., 2003); and (iii) the simultaneous inactivation of the ApoAI gene by crossing SR-BI−/− with ApoAI−/− (yielding double KO mice), which resulted in a large reduction of total plasma cholesterol without a concomitant reduction of the abnormally large HDL particle size.

Finally, Yesilaltay et al. (2006) used transient adenovirus-mediated and stable transgenic expression of both wild-type SR-BI and the double point mutation of the SR-BI receptor (SR-BI RR), which binds LDL and abnormally large HDL but not normal HDL, to induce hepatic expression of SR-BI in SR-BI−/− mice. Hepatic transgenic SR-BI expression in SR-BI−/− female mice resulted in reduced plasma total cholesterol levels, increased biliary cholesterol secretion and restored fertility. Fertility was also restored in SR-BI−/− female mice with overexpression of SR-BI RR. In both sets of experiments, normalization of HDL size and UC:TC ratio occurred. Collectively, these studies establish the mechanism of preimplantation embryo arrest in female SR-BI KO mice to be an aberrant control of hepatic and plasma HDL lipoprotein metabolism with abnormal lipid composition (i.e. UC:TC ratio) and large HDL particle size, rather than a lack of SR-BI expression in the ovary (Attie, 2006).

**Murine ABCA1 KO model**

Further evidence for the important role of HDL in reproduction is provided by the homozygous female ABCA1-deficient mouse which suffers from reduced fecundity, reduced litter size and decreased numbers of pregnancies secondary to reduced HDL cholesterol levels (Christiansen-Weber et al., 2000; Aiello et al., 2003). As discussed previously, ABCA1 transporter protein is a key enzyme involved in reverse cholesterol transport critical to normal HDL metabolism (Lawn et al., 1999). HDL maturation is markedly reduced in this model with significant alterations in phospholipid composition (~80% decrease in phosphatidylycerol) (Francone et al., 2003).

Familial HDL deficiency, also known as Tangier disease, produces accelerated atherosclerosis (Bodzioch et al., 1999; Brooks-Wilson et al., 1999; Remaley et al., 1999; Rust et al., 1999; Brousseau et al., 2000). In several pedigrees of familial HDL deficiency, there is evidence for fertility in women suffering from homozygous ABCA1 mutations, which results in substantially reduced plasma HDL cholesterol levels (1–2 mg/dl). The latter suggests that HDL cholesterol is not obligatory for reproductive potential in the human female, but may play a role in reproductive aging and oocyte health (Bodzioch et al., 1999). However, it is now known that other ABC transporter proteins such as ABCG1 and ABCG4 may also be involved in reverse cholesterol transport from cells, which could mitigate the effects of reduced or absent ABCA1 expression (Wang et al., 2004; Kennedy et al., 2005). Whether HDL cholesterol is directly influencing oocyte quality via regulatory mechanisms involving cumulus granulosa cells, within the follicle, is not known.

**Restricted ovulator chicken mutant model**

In the chicken, the nutrients in the yolk of oocytes are predominantly derived from plasma VLDL and vitellogenin via receptor-mediated endocytosis involving the 95-kDa oocyte vitellogenesis receptor (OVR). The OVR is a homologue to the mammalian VLDL receptor. Hens of the restricted—ovulator (R/O) chicken strain carry a naturally-occurring single mutation of the OVR (Bujo et al., 1994, 1995a, b). These hens display severe hyperlipidemia characterized by 3- to 6-fold increases in serum triglycerides, cholesterol and phospholipids with associated premature atherosclerosis (Ho et al., 1974). The R/O mutation leads to greatly reduced OVR expression on the oocyte surface while somatic cells demonstrate a differentially-spliced variant absent from germ cells. Oocytes from these hens do not reach maturation, become atretic and fail to ovulate leading to sterility via failure to lay eggs (Nimpf et al., 1989). Although carrier roosters of the mutant strain do not show any reproductive abnormalities, the homozygous state has not been studied (Ho et al., 1974).

There are substantial differences between avian and mammalian lipoprotein metabolism as well as oocyte development and structure; hence, the mechanisms leading to female infertility in the chicken model may not be similar to those in the SR-BI KO mouse. However, it is interesting to note that chicken yolk is similar to mammalian FF, in that it contains HDL particles which are principally derived from plasma (Vieira et al., 1995). In these studies conducted prior to the discovery of SR-BI, the transport mechanism of HDL components into the yolk was not identified. However, this transport mechanism was shown to be distinct from other yolk lipoproteins and governed by ‘post-endocytic modification of the lipid moiety of HDL’ or ‘receptor-independent fluid-phase uptake’ rather than receptor-mediated endocytosis (Vieira et al., 1995). The eventual discovery of SR-BI-mediated selective lipid uptake from HDL (Acton et al., 1996) is consistent with the plasma to yolk HDL transport described by Vieira et al. (1995). The subsequent demonstration that SR-BI is expressed by avian liver (Duggan et al., 2002) makes SR-BI a possible candidate responsible for HDL component transport into the chicken yolk.

**The liver X receptor KO model**

Intracellular cholesterol metabolism is regulated in part through the expression of ABCA1 transporter proteins via the liver X receptor (LXR) (Volle and Lobaccaro, 2007). LXRs are important regulators of cholesterol, fatty acid and glucose homeostasis (Steﬀenssen and Gustafsson, 2004). In adrenal tissues, LXRα and LXRβ contribute to the maintenance of the steroidogenic basal state, preventing accumulation of unesterified cholesterol by targeting genes controlling cholesterol storage, efflux and steriodogenesis (Cummins et al., 2006). LXRx functions to limit the concentration of intracellular unesterified cholesterol by coordinate up-regulating genes involved in cholesterol efflux (ABCA1) and storage (ApoE and SREBP-1c), and down-regulating steriodogenic acute regulatory gene expression (Cummins et al., 2006). Deficiency of LXR induces adrenomegaly, cholesterol accumulation and excessive steriodogenesis in adrenal tissues (Cummins et al., 2006).

In support of their steriodogenic activities, the granulosa and theca cells of the ovarian follicle acquire large amounts of CEs which are hydrolyzed to unesterified cholesterol. During the peri-ovulatory
period, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) stimulate granulosa cells to synthesize progesterone, which subsequently inhibits cholesterol esterification (Warner et al., 1995). Excessive intracellular unesterified cholesterol cannot be stored, and must therefore be excreted in order to avoid accumulation in the plasma membrane, which may lead to cellular dysfunction. Granulosa cells have recently been shown to express substantial levels of LXRs, principally LXR\(_B\), relative to other tissues (Drouineaud et al., 2007). LXR agonist stimulation of luteinizing granulosa cells induces ABCA1, ABCG1, PLTP and ApoE expression with a consequent increase in the capacity for cellular cholesterol efflux (Drouineaud et al., 2007). Theca interna cells express ABCA1 transporter mRNA; however, LXR expression studies have yet to be reported (Wu et al., 2003). LXR is expressed in mouse oocytes, and LXR KO mice (LXR\(^{−/−}\)) conceive less frequently and have significantly fewer pups than wild-type mice (Steffensen et al., 2006). An LXR agonist, GW3965, induced nude, but not cumulus-enclosed, oocytes, to undergo germinal vesicle breakdown (GVBD) in wild-type mice. In contrast, LXR\(^{−/−}\) oocytes underwent GVBD only in response to zymosterol, a C-27 unsaturated sterol with meiosis-activating sterol activity (Steffensen et al., 2006). These data suggest that oocyte nuclear maturation via LXR-ligand induction of GVBD is, at least in part, responsible for the reduced fecundity in LXR\(^{−/−}\) female mice. However, the effects of LXR on ABCA1 expression in wild-type cumulus oocyte complexes raise the question of cholesterol homeostasis alterations in this model. In addition to hypo-fertile females, the LXR\(^{−/−}\) KO mouse model produces pronounced infertility in males characterized by degeneration of the testes due to excessive accumulation of cholesterol, severe cellular disruption and the dysregulation of spermatogenesis (Frenoux et al., 2004).

**Hypothesized role of HDL-dependent cholesterol efflux in reproduction**

The work by Krieger and colleagues (Trigatti et al., 1999; Miettinen et al., 2001; Yesilaltay et al., 2006) points to abnormal HDL particles as the basis for infertility due to embryo arrest in the SR-BI KO mice, which is not seen in other mutant models of HDL metabolism. All of the models previously discussed share defects that impair cholesterol efflux to HDL, disrupting normal intra-follicular HDL metabolism. The infertility described in each of the models (SR-BI\(^{−/−}\), ABCA1\(^{−/−}\), R/O chicken and LXR\(^{−/−}\)) could be explained by the lack of a functional cholesterol acceptor (abnormal HDL in SR-BI\(^{−/−}\) or R/O chicken), a transporter (ABCA1\(^{−/−}\)) or a transporter regulator (LXR\(^{−/−}\)). The resulting cholesterol efflux deficiencies may disrupt intra-follicular cholesterol homeostasis and consequently disturb normal oocyte development.

The presence of LXR and ABCA1 in cumulus oocyte complexes (Steffensen et al., 2006), together with impaired reproduction in their absence, suggests an important role for a functional HDL cholesterol acceptor mediating cholesterol homeostasis via an efflux mechanism. Efficient cholesterol efflux is principally dependent upon the binding between HDL particles and the ABCA1 transporter with a small contribution from SR-BI–HDL interactions. ABCA1 reacts most efficiently with small, lipid-poor, pre-\(β\)-HDL (Asztalos et al., 2005) and depletion of pre-\(β\)-HDL by chymase inhibits ABCA1-mediated cholesterol efflux (Favari et al., 2004). In SR-BI KO mice, the HDL particles are large ApoAI-containing particles which may not participate as efficient cholesterol acceptors (Rigotti et al., 1997a, b). These abnormal HDL particles appear to be a poor substrate for LCAT, adversely affecting the conformation of ApoAI and decreasing its ability to activate LCAT (Ma et al., 2005; Yesilaltay et al., 2006; Lee et al., 2007).

Given the absence of the ABCA1 transporter, we might also expect the ABCA1 KO to be completely infertile; however, the presence of the recently identified ABCG1 and ABCG4 transporters may permit sufficient cholesterol efflux activity to allow for partial female reproductive potential, albeit with subsequent embryonic developmental deficits (Christiansen-Weber et al., 2000; Wang et al., 2004). Although ABCG1 and ABCG4 are present together with ABCA1 in the SR-BI KO, we hypothesize that the lack of an acceptable HDL cholesterol acceptor may be a fundamental problem in the SR-BI KO. In the R/O chicken with severe hyperlipidemia, it is possible that altered cholesterol efflux may be contributing to the phenotype. Collectively, these mutant models support the role of HDL concentration, structure and composition in determining mammalian female reproductive potential.

**Clinical importance of HDL metabolism in reproduction**

**Embryo morphology to assess HDL effects on oocyte quality**

During the course of our own studies, we recognized the need to identify appropriate outcome variables to correlate with serum and FF HDL components. Although morphologic parameters of early human embryo development during in vitro fertilization (IVF) have been assessed at various time points, including zygote and early cleavage (EC) stages (Säkkas et al., 1998; Scott and Smith, 1998; Manor et al., 1999; Tesarik and Greco, 1999; Wittmer et al., 2000; Bos-Mikich et al., 2001; Lundin et al., 2001; Ebner et al., 2003; Neuber et al., 2003; Salumets et al., 2003; Van Montfoort et al., 2004; Ciray et al., 2005; Hesters et al., 2008), we chose to study Day 3 embryo morphology markers, assessing embryo fragmentation separately from embryo cell cleavage number.

The well-recognized dependence of IVF implantation success on maternal age is principally thought to be due to decreased oocyte competence, as evidenced by increased aneuploidy and embryonic arrest with increasing age (Pantos et al., 1999; Pellestor et al., 2006); however, it is known that human embryos demonstrate variable degrees of cytoplasmic fragmentation and mitotic activity in vitro. It has been postulated that embryo fragmentation is a phenomenon resulting from abnormal cytokinesis (Alkani et al., 2005). Follicular size has been recently demonstrated to correlate with oocyte competence based on the degree of embryo fragmentation and intra-cytoplasmic sperm injection fertilization rates (Ectors et al., 1997; Bergh et al., 1998; Rosen et al., 2008). It has been suggested that embryo fragmentation is an indicator of apoptosis (Jurisicova et al., 1995, 1996), but that relationship is controversial (Antczak and Van Blkem, 1999).

A well-established association inversely correlates the degree of embryo fragmentation and the likelihood of blastocyst formation and
implantation (Steer et al., 1992; Giorgetti et al., 1995; Ziebe et al., 1997; Rijdors and Jansen, 1998; Alikani et al., 1999, 2000; Desai et al., 2000; Ebner et al., 2001; Hardy et al., 2003; Volpes et al., 2004; Stone et al., 2005; della Ragione et al., 2007). Although other embryo morphologic parameters such as blastomere symmetry and multinucleation have been correlated with embryo quality, chromosomal abnormalities and implantation rate (Tesarik et al., 1987; Kligman et al., 1996; Laverge et al., 1997; Jackson et al., 1998; Hardarson et al., 2001; Van Royen et al., 2003), embryo cleavage rate and the degree of cytoplasmic fragmentation remain the clinical laboratory biomarkers attributed the greatest importance in predicting embryo viability during the earliest stages of human development (Alikani et al., 2000). Thus, we conducted our own studies using embryo cell number and fragmentation score which we considered the optimal outcome parameters to assess the effects of HDL parameters on human oocyte health.

**Peri-follicular vascularity and the blood–follicular barrier**

Peri-follicular vascularity and follicular basal lamina properties influence the presence of HDL particles in FF. The blood–follicular barrier including the follicular basement membrane acts as a dynamic ‘sieve’ permitting selective diffusion of various proteins and small molecules based on size and charge, including ApoAI, ApoAII, ApoAIV and haptoglobin. The follicular basement membrane acts as a dynamic ‘sieve’ (Le Goff, 1994). Theoretical calculations indicated that FF HDL is a filtration product from plasma; however, HDL particles may also undergo intra-follicular metabolic transformations which have been linked to hormonal synthesis and reverse cholesterol transport. It has been reported that human follicles contained HDL particles which were cholesterol-poor relative to serum HDL particles, but significantly (P < 0.05) richer in phospholipids when expressed as per cent weight (i.e. 28.5% in FF versus 25.0% in serum) (Jaspar et al., 1996, 1997).

Our clinical analyses of FF and serum HDL concentration and composition confirm differences in absolute HDL component levels and lipid compositions with FF HDL particles being proportionally cholesterol-poor and phospholipid-rich compared with serum (Browne et al., 2008). These results are consistent with earlier described findings that FF HDL contains a higher proportion of smaller LDL-sized particles relative to larger LDL-sized particles (Le Goff, 1994; Jaspar et al., 1996). We have also reported lower absolute HDL particle concentrations (i.e. ApoAl levels) in FF compared with serum; however, levels between the compartments were correlated in a statistically significant (P < 0.05) fashion (Table II) (Browne et al., 2008). Thus, it appears that FF HDL is at least partially blood-derived.

Our examination of the proportional HDL particle composition in FF and serum specimens provides a more interesting picture than that observed employing absolute particle composition. Previous studies indicated that ApoAI molecules in FF HDL particles are not different from blood plasma (i.e. each is ~42% ApoAl by mass) (Jaspar et al., 1997). We have demonstrated that FF ApoAl correlates with FF HDL cholesterol in a statistically significant fashion (r = 0.47, P = 0.000, n = 60) (Browne et al., 2008). After standardizing HDL concentration per molecule of ApoAl (i.e. HDL component concentration divided by ApoAl concentration), it was evident that the FF HDL core was ~25% lower in CE and 40% lower in triglycerides, whereas the surface was 10% higher in phospholipids and 40% higher in unesterified cholesterol, as compared with serum HDL.

**Follicular fluid and HDL**

In contrast to extravascular fluids that contain LDL and VLDL such as lymph and interstitial fluid (Sloop et al., 1987; Reichl, 1990), Simpson et al. (1980) demonstrated that human FF contained HDL as its sole lipoprotein. Others have confirmed the near exclusiveness of HDL in FF, further noting the alterations of several FF HDL lipid parameters compared with plasma (Perret et al., 1985; Volpe et al., 1991). Bovine follicles exhibit the same exclusive HDL content with an increase in HDL-related lipid levels associated with follicular maturation (Brantmeier et al., 1987). Since the blood–follicle barrier is permeable to serum proteins up to 300 kDa in size, larger HDL, LDL and VLDL particles are not expected to enter FF from plasma (Shalgi et al., 1973). Rather, only small HDL particles (HDL₃) would be expected to enter FF. However, a recent study has raised uncertainty about the exclusivity of HDL particles in FF with the detection of VLDL-, intermediate-LDL- and LDL-sized particles (Von Wald et al., 2009). Prior reports suggest that FF HDL particle size and composition may result from selective porosity of the follicle barrier as well as metabolic remodeling events occurring within FF (Le Goff, 1994; Jaspar et al., 1997).

Examination of HDL in mare plasma and FF has identified increased cholesterol/phospholipid and esterified/unesterified cholesterol molar ratios in FF HDL compared with plasma HDL (Le Goff, 1994). Furthermore, a decrease in FF HDL particle size diameters (4.2–9.2 nm) was reported when compared with plasma HDL (5.5–9.5 nm) (Le Goff, 1994). Theoretical calculations indicated that FF HDL is a filtration product from plasma; however, HDL particles may also undergo intra-follicular metabolic transformations which have been linked to hormonal synthesis and reverse cholesterol transport. It has been reported that human follicles contained HDL particles which were cholesterol-poor relative to serum HDL particles, but significantly (P < 0.05) richer in phospholipids when expressed as per cent weight (i.e. 28.5% in FF versus 25.0% in serum) (Jaspar et al., 1996, 1997).

Our clinical analyses of FF and serum HDL concentration and composition confirm differences in absolute HDL component levels and lipid compositions with FF HDL particles being proportionally cholesterol-poor and phospholipid-rich compared with serum (Browne et al., 2008). These results are consistent with earlier described findings that FF HDL contains a higher proportion of smaller LDL-sized particles relative to larger LDL-sized particles (Le Goff, 1994; Jaspar et al., 1996). We have also reported lower absolute HDL particle concentrations (i.e. ApoAl levels) in FF compared with serum; however, levels between the compartments were correlated in a statistically significant (P < 0.05) fashion (Table II) (Browne et al., 2008). Thus, it appears that FF HDL is at least partially blood-derived.
(Browne et al., 2008). These observations indicate that the differences in absolute lipid levels in FF may be due to a lower number of HDL particles as well as differences in the lipid compositions of HDL particles. These differences suggest that FF HDL particles are depleted of core CEs and enriched in surface phospholipids relative to plasma HDL particles.

### HDL particle and the human embryo

Given the evidence from the SR-BI KO mouse model, we chose to examine the role of serum and FF HDL particles on the reproductive potential of the human oocyte using embryo morphologic parameters as surrogate biomarkers for oocyte quality during IVF. Our findings demonstrated negative correlations between FF HDL cholesterol and ApoAI levels with embryo fragmentation evaluated after 72 h of IVF culture (Fig. 3 and Table III) (Browne et al., 2008). However, no statistically significant correlations were detected between HDL cholesterol and ApoAI levels and embryo cell cleavage number, adjusted for day of transfer (Browne et al., 2008). The negative association between FF HDL cholesterol and embryo fragmentation is interesting in light of the absence of any correlation with embryo cell cleavage rate (Table III). Our findings support the premise that FF HDL particles are depleted of core CEs and enriched in surface phospholipids relative to plasma HDL particles.

In contrast to the SR-BI-KO mouse model, we have not detected associations between embryo cell cleavage rates during IVF and any of the HDL lipid components (Trigatti et al., 1999; Browne et al., 2008). We had hypothesized an effect of HDL cholesterol on embryo cleavage rate rather than embryo fragmentation given the high percentage of embryo cell cleavage arrest seen with abnormal HDL particles in the SR-BI KO model (Trigatti et al., 1999). Rather, the rate of embryo cell cleavage appears to be related to the oxidative state of the follicle as we have demonstrated positive correlations between embryo cell number adjusted for day of transfer and HDL particles.

![Figure 3](image)

**Figure 3** Embryo fragmentation score as a function of unadjusted FF concentrations of HDL particle cholesterol and ApoAI. HDL, high-density lipoprotein; ApoAI, apolipoprotein AI. Pearson’s correlation coefficients are provided in the legend (n = 30). Embryo fragmentation score was defined as: grade 1, 0% fragmentation; grade 2, 1–10% fragmentation; grade 3, 11–25% fragmentation; grade 4, 26–50% fragmentation; and grade 5, 51% or greater fragmentation. Adapted from data presented in Browne et al. (2008), Oxford Journals, Copyright 2008.

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**Table II** Distributions of HDL particle lipids and enzymes in paired serum and FF specimens

<table>
<thead>
<tr>
<th></th>
<th>Serum median, mg/dl (IQR)</th>
<th>FF median, mg/dl (IQR)</th>
<th>Median difference, mg/dl (P-value)</th>
<th>Correlation (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (n = 59)</td>
<td>37.2 (12.4)</td>
<td>20.9 (6.0)</td>
<td>17.3 (&lt;0.0001)</td>
<td>0.65 (&lt;0.0001)</td>
</tr>
<tr>
<td>Phospholipids (n = 40)</td>
<td>94.6 (12.1)</td>
<td>75.5 (12.0)</td>
<td>20.5 (&lt;0.0001)</td>
<td>0.60 (&lt;0.0001)</td>
</tr>
<tr>
<td>Triglycerides (n = 39)</td>
<td>8.4 (4.5)</td>
<td>6.7 (5.5)</td>
<td>0.84 (0.546)</td>
<td>0.09 (0.597)</td>
</tr>
<tr>
<td>ApoAI (n = 59)</td>
<td>157.3 (23.0)</td>
<td>105.5 (20.8)</td>
<td>51.0 (&lt;0.0001)</td>
<td>0.44 (0.001)</td>
</tr>
<tr>
<td>PON1-arylesterase (n = 60)</td>
<td>309.7 (99.8)</td>
<td>186.4 (66.3)</td>
<td>114.2 (&lt;0.0001)</td>
<td>0.74 (&lt;0.0001)</td>
</tr>
<tr>
<td>PON1-paraoxonase (n = 60)</td>
<td>186.5 (126.5)</td>
<td>116.4 (81.2)</td>
<td>59.7 (&lt;0.0001)</td>
<td>0.95 (&lt;0.0001)</td>
</tr>
<tr>
<td>PON2-simvastatinase (n = 58)</td>
<td>10.6 (3.6)</td>
<td>16.5 (2.8)</td>
<td>−5.6 (&lt;0.0001)</td>
<td>0.36 (0.006)</td>
</tr>
</tbody>
</table>

Wilcoxon’s signed rank and Spearman’s correlation coefficients between serum and FF specimens. Sample sizes vary due to limited sample volume for all analyses in some paired specimens. HDL, high-density lipoprotein; ApoAI, apolipoprotein AI; IQR, interquartile range (75th–25th percentile); PON, paraoxonase. Adapted from data presented in Browne et al. (2008), Oxford Journals, Copyright 2008.
Table III: Spearman rank correlation coefficients (P-values) between HDL components and embryo quality indicators

<table>
<thead>
<tr>
<th></th>
<th>Embryo fragmentation score (n = 30)</th>
<th>Embryo cell number (n = 31)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>−0.53 (0.003)</td>
<td>−0.11 (0.565)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>−0.22 (0.336)*</td>
<td>−0.23 (0.337)*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.01 (0.977)*</td>
<td>−0.12 (0.621)*</td>
</tr>
<tr>
<td>ApoAl</td>
<td>−0.50 (0.005)</td>
<td>−0.04 (0.842)</td>
</tr>
<tr>
<td>PON1-arylesterase</td>
<td>−0.39 (0.032)</td>
<td>0.09 (0.613)</td>
</tr>
<tr>
<td>PON1-paraoxonase</td>
<td>0.17 (0.366)</td>
<td>0.04 (0.825)</td>
</tr>
<tr>
<td>PON3-simvastatinase</td>
<td>−0.25 (0.192)</td>
<td>0.10 (0.603)</td>
</tr>
<tr>
<td>Follicular fluid</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol</td>
<td>−0.59 (0.001)</td>
<td>0.02 (0.896)</td>
</tr>
<tr>
<td>Phospholipids</td>
<td>−0.23 (0.319)*</td>
<td>0.14 (0.556)*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>0.04 (0.862)*</td>
<td>−0.20 (0.408)*</td>
</tr>
<tr>
<td>ApoAl</td>
<td>−0.54 (0.002)</td>
<td>0.14 (0.445)</td>
</tr>
<tr>
<td>PON1-arylesterase</td>
<td>−0.20 (0.280)</td>
<td>0.29 (0.122)*</td>
</tr>
<tr>
<td>PON1-paraoxonase</td>
<td>0.15 (0.438)</td>
<td>0.07 (0.719)</td>
</tr>
<tr>
<td>PON3-simvastatinase</td>
<td>0.20 (0.308)*</td>
<td>−0.11 (0.585)*</td>
</tr>
</tbody>
</table>

Sample sizes vary for certain analytes due to limited FF sample volume; correlations with P < 0.05 in bold typeface. HDL, high-density lipoprotein; ApoAl, apolipoprotein Al; PON1, paraoxonase. Adapted and reprinted from Browne et al., (2008), Oxford Journals, Copyright 2008.

*aStatistically significant positive association during ordinal logistic regression modeling of 22 Day 3 embryos (β = 0.08, P = 0.02).

Covariates of importance. As FF ApoAl level serves as a biomarker for HDL particle number (Fruchart and Ailhaud, 1992), our findings suggest that the number of HDL particles present in FF may be physiologically important within the mature pre-ovulatory follicle. The co-existing associations between both HDL cholesterol and ApoAl and embryo fragmentation underscore the importance of the FF HDL particle as a determinant of embryo fragmentation in vitro. Although ApoAl comprises 40–50% of the HDL particle mass, HDL particles are, by and large, heterogeneous in structure, represented by both lipid-poor and lipid-rich content with varying discoidal and spherical shapes as discussed previously (Segrest et al., 2000a, b; Klon et al., 2002; Jia et al., 2005; Curtiss et al., 2006; Silva et al., 2008). Little is currently known about the size and structure of HDL particles within FF; however, our data comparing relative proportions of cholesterol and other lipids within serum and FF suggest that significant remodeling of HDL particles occurs within the ovarian follicle. As we have not identified significant associations between other lipid components of the HDL particle such as HDL triglyceride or phospholipids levels and embryo morphology parameters (Table II) (Browne et al., 2008), it is yet unclear as to whether and how the constitution of the HDL particle may be important in influencing embryo morphology. Since it is now recognized that FF HDL particles are cholesterol-poor and phospholipid-rich relative to blood, further studies are needed to better understand intra-follicular HDL particle remodeling and cholesterol metabolism, with further assessment of their impact on the cumulus complex. In contrast to the role of lipoprotein-derived cholesterol as a precursor for corpus luteal steroidogenesis (Christenson and Devoto, 2003), the importance of cholesterol metabolism and specifically the HDL particle within the human ovarian follicle is not well understood during folliculogenesis. We hypothesize that the relationship between HDL cholesterol and embryo fragmentation reflects alterations in intracellular cholesterol homeostasis within the oocyte.

One possible mechanism for the effect of cholesterol on follicular cells relates to lipid raft dynamics. Intracellular cholesterol and sphingolipids play important roles in cytokinesis as constituents of ‘lipid rafts’ (Simons and Ikonen, 2000). Lipid rafts are microenvironment lipid structures within the cellular plasma membrane serving a number of various cellular functions including signal transduction (Simons and Toomre, 2000). Lipid rafts are important for the precise control of cholesterol homeostasis within cells (Simons and Ikonen, 2000) and several studies point toward a function for lipid rafts in oocyte development (Emoto et al., 1996, 2005; Emoto and Umeda, 2000). Preceding the cleavage furrow of the cytokinetic event during cell division, a reorganization of the cholesterol-rich lipid raft formations occurs leading to the formation of the actin-based contractile ring that facilitates division (Ng et al., 2005). In the Xenopus model, lipid raft cholesterol depletion of oocytes using methyl-β-cyclodextrin reduces oocyte meiotic maturation (Buschiazzo et al., 2008). Membrane-associated cholesterol raft domains within the oocyte also appear to be critically important for the tyrosine-phosphorylated events of sperm fertilization (Sato et al., 2002). Cholesterol lipid rafts within the mammalian oocyte may be important for early embryo cleavage (Comiskey and Warner, 2007). In the cleaving embryo, cholesterol-rich lipid rafts are found in abundance adjacent to the cleavage furrow that occurs with cytokinesis (Comiskey and Warner, 2007). Cholesterol depletion blocks the completion of cytokinesis in sea urchin embryos (Ng et al., 2005). Studies in various cell lines detail a regulatory role for cholesterol with persistent depletion resulting in G2 arrest (Martinez-Botas et al., 1999).

Oocytes do not accumulate intracellular cholesterol via receptor-mediated selective uptake as they have neither SR-BI nor LDL cholesterol receptors (Trigatti et al., 1999; Sato et al., 2003). Furthermore, the developing mammalian oocyte does not have the ability to synthesize cholesterol de novo based on the absence of transcripts encoding cholesterol synthesis pathway enzymes (Su et al., 2008). Rather, cholesterol appears to be transported into the oocyte through a coordinated mechanism requiring cholesterol biosynthesis in cumulus granulosa cells that involves signaling by the oocyte through bone morphogenetic protein 15 (BMP-15) and growth differentiation factor 9 (GDF-9) (Su et al., 2008).

Cholesterol synthesis may not be the only mechanism by which cumulus cells accumulate intracellular cholesterol. Although it was previously reported that SR-BI mRNA expression was limited to theca cells prior to the LH surge (Li et al., 1998), SR-BI expression has been recently demonstrated from wild-type uniluteized (i.e. pre-LH surge) cumulus cells, suggesting the potential for direct cholesterol transport into cumulus cells during folliculogenesis via HDL (Su et al., 2008). However, as previously discussed, the resumption of reproductive potential in the SR-BI KO model following...
transplantation of SR-BI−/− ovaries into ovariectomized SR-BI+/+ females argues against an important role for selective lipid uptake into cumulus cells via SR-BI receptors during folliculogenesis (Miettinen et al., 2001). Therefore, it is interesting to postulate that reverse cholesterol transport from cumulus cells to HDL particles, via ABCA1, ABCG1 or ABCG4 transporters in cumulus cells, may be important in influencing cholesterol homeostasis in both cumulus cells and oocytes.

There is evidence that the preimplantation embryo does not have active cholesterol biosynthesis capacity due to a lack of hydroxy-methylglutaryl co-enzyme A reductase activity until the blastocyst stage; thus, the cholesterol required for early embryo development appears to be solely reliant on the intracellular cholesterol levels present in the oocyte prior to fertilization (Pratt, 1982, 1985). The delay seen in preimplantation development of double mutant embryos for BMP-15 and GDF-9 may be a result of a deficiency in cholesterol metabolism (Su et al., 2004). Thus, our observations pose the questions of whether FF HDL cholesterol plays a modulatory role on the intracellular cholesterol content of cumulus cells and the oocyte, and whether the negative association with embryo fragmentation we have observed occurring during embryo cell cleavage is a direct reflection of this relationship.

**Summary and future directions**

In this narrative review, we have summarized the current understanding of HDL metabolism with a focus on cholesterol transport between cells and the association of HDL particle composition with respect to reproduction in various models, including the human. Although much is known about the role of HDL in cardiovascular health and disease, we have only recently begun to understand the relationship between HDL particles and the reproductive system. The remarkable study by Trigatti et al. (1999) provided the first clue to an important relationship that exists between HDL particles and the ovarian follicle. Our clinical observations have confirmed the need to better understand the roles of HDL and cholesterol metabolism within FF on oocyte development and competence.

Current technology using NMR lipoprotein spectrometry will allow further elucidation of the HDL particle types present in FF and the dynamic remodeling that may occur within the follicle to potentially influence oocyte and granulosa cell development (Jeyarajah et al., 2006; Jiang et al., 2008; van der Graaf et al., 2008). We hypothesize that reverse cholesterol transport within the developing follicle may be critical to normal oocyte development and emphasize the need for further studies to elucidate the mechanisms that regulate intracellular cholesterol transport during folliculogenesis. The collective information garnered from our current and future studies will further the identification of clinical biomarkers of HDL metabolism that may reliably predict IVF outcomes and offer potential novel targets for adjuvant therapy to improve gonadotrophin-stimulated folliculogenesis during IVF.

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