Journal of Assisted Reproduction and Genetics (2019) 36:1657–1664
https://doi.org/10.1007/s10815-019-01523-9

ASSISTED REPRODUCTION TECHNOLOGIES

HDL functionality in follicular fluid in normal-weight and obese women undergoing assisted reproductive treatment

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Received: 19 March 2019 / Accepted: 8 July 2019 / Published online: 23 July 2019

Abstract

Objective High-density lipoproteins (HDL) exert pleiotropic roles in follicular fluid (FF). Previous studies have reported a relationship between obesity, infertility, and systemic oxidative stress. The aim of our study was to investigate for the first time the HDL functional properties in FF in obesity.

Methods In this observational study, overweight/obese ($n = 20$) and normal-weight women ($n = 38$) undergoing assisted reproductive technology were included. Compositional properties and biochemical marker of functionality (HDL oxidation rate), HDL-associated antioxidants (paraoxonase-1 activities and CoQ10 content), and lipid hydroperoxide levels were evaluated in FF from normal-weight and overweight/obese women. Correlations between biochemical parameters and indices for oocyte and embryo quality were studied.

Results FF-HDL obtained from overweight/obese women are characterized by high intrinsic ability to be oxidized compared with FF-HDL from normal-weight women. These alterations are associated with lower activities of paraoxonase-1 (PON1), higher levels of lipid peroxidation, and a lower total antioxidant capacity in FF. Moreover, an association between PON1 activity and FF-HDL oxidation and clinical parameter of oocyte quality was observed.

Conclusion Our data suggest that the quality of FF-HDL is important determinant for oocyte quality. Therefore, targeting FF-HDL functionality, in addition to FF-HDL-C levels, may represent a promising and interesting biomarker for reproductive outcomes.

Keywords High-density lipoproteins · Paraoxonase-1 · Obesity · Oxidative stress · Follicular fluid · Assisted reproductive treatment

Introduction

Obesity is associated with a higher risk for cardiovascular disease that has been related to alteration of plasma lipoproteins and to an unbalance between free radicals and antioxidant defense [1–5]. Obesity is also associated with impaired reproductive functionality, decreased fecundity [6, 7], and increased pregnancy complications [8, 9]. Furthermore, previous studies have demonstrated that obese women undergoing assisted reproductive technology (ART) procedures have lower success rate compared with normal-weight patients [10, 11]. Previous studies have demonstrated that the biochemical composition of follicular fluid (FF) exerts a key role in the efficiency of ART techniques [12, 13]. The FF fluid is primarily composed of proteins, steroid hormones, and lipids that accumulate within the oocyte and help its differentiation [14]. Modifications in lipid and protein composition of the FF of obese women undergoing assisted reproductive treatment have been reported to be correlated to lower oocyte number and poorer embryo quality [15]. In our previous study, we demonstrated that obesity and visceral fat deposit were significantly related with a lower number and quality of oocyte and embryo in women undergoing ART procedures [16].

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Regarding the lipoprotein composition, follicular fluids are characterized by a major component of high-density lipoproteins (HDL) that largely exceed other lipoproteins [17]. In blood, HDL exert an antioxidant and anti-inflammatory role due to the enzyme paraoxonase-1 (PON1). The presence of HDL in the FF is influenced by the follicular basal lamina and by perifollicular vascularity [18]. Previous studies have shown that HDL contribute to the intra-follicular homeostasis of cholesterol and have a role in embryo development [18–20]. It has been also proposed that antioxidant molecules in FF could play a role in maintaining the control of the redox balance of follicular fluid which is essential for a correct oocyte maturation. Among antioxidants, a key role has been proposed for HDL-associated PON1 [21]. In fact, Meijide et al. have shown a positive correlation between PON1 arylesterase activity and paraoxonase activity and total number of retrieved oocytes, thus providing indirect evidence for the role of PON in follicle maturation [21].

Among low molecular weight antioxidants embedded in lipoproteins, CoQ10 plays a critical role being the major endogenous lipophilic antioxidant produced by the organism. Content of CoQ10 in HDL in the FF has been related to oocyte fertilization and embryo grading [22].

The aim of our study was to investigate for the first time the HDL composition and functional properties in FF of overweight/obese and normal-weight women undergoing fertility treatments. Therefore, we evaluated biochemical markers of HDL functionality (FF-HDL oxidation rate), HDL-associated antioxidants (namely PON1 enzymatic activities and CoQ10 content), and lipid hydroperoxide levels in FF from normal-weight and overweight/obese women. Correlations between biochemical parameters and indices for oocyte and embryo quality were also studied.

Methods

Patients and follicular fluid collection

Fifty-eight women undergoing in vitro fertilization and embryo transfer (IVF–ET) program at the Centre for Assisted Reproduction (Woman's Health Sciences Department, Gynaecologic Section, Polytechnic University of Marche, Ancona, Italy) were included in this study.

This observational study was approved by the Departmental Review Board and Local Ethical Committee, the full information about the current study was given to each patient, and the written consent was obtained before the procedure. The study was conducted in accordance with the Declaration of Helsinki.

For each patient, anamnestic and biometrical characteristics including age, obstetrical and gynecological history, ovarian reserve (AMH – anti-Mullerian hormone, basal FSH-follicle stimulating hormone, antral follicle count), height, and weight were collected. The women were subdivided into three groups, according to their body mass index (BMI): normal weight (defined as BMI ≥ 18.5 and ≤ 24.9 kg/m^2), overweight (defined as BMI ≥ 25 and ≤ 29.9 kg/m^2), and obese (defined as BMI ≥ 30 and < 35 kg/m^2) (Table 1). Patients with BMI > 35 were excluded from IVF treatment protocol. Both groups matched for age and ovarian reserve parameters.

Long stimulation protocol (Leuprolide Acetate, Recombinant FSH, hCG 10.000 UI ovulation trigger) for controlled ovarian stimulation was adjusted for each patient and respected individual indications [23].

During controlled ovarian stimulation, every woman was monitored using transvaginal ultrasounds and when three or more follicles reached a diameter of ≥ 18 mm, hCG was administered. Transvaginal ultrasound–guided oocyte retrieval was then performed within 36 h. Samples of follicular fluid (FF) were obtained from all major follicles (> 18 mm) bilaterally, during the oocyte retrieval procedure performed by transvaginal ultrasound–guided follicular aspiration system (COOK 17 g/35-cm needle). Samples of FF were obtained during the oocyte retrieval procedure using a transvaginal follicular aspiration. Samples were immediately centrifuged for 10 min at 2080 rpm; subsequently, the supernatant was taken and stored at − 80 °C and used for the biochemical analysis.

Standard IVF/ICSI procedures were performed as described by Prados et al. [24]. Different parameters as markers for oocyte and embryo quality have been evaluated as previously reported [16] such as the number of oocytes retrieved; the number of "good quality oocytes" according to Lin et al. [25]; the fertilization rate (number of two-pronuclear zygotes (2PN)/number of oocytes, following IVF or ICSI on day 1); the percentage of embryos obtained (number of embryos/number of oocytes); and the number of embryos transferred in womb.

Composition of follicular fluids

Lipids HDL-cholesterol (HDL-C) and triglycerides levels were measured in FF samples using commercially available kits (Roche Diagnostics, Switzerland). Total FF protein content has been evaluated according to Bradford [26].

Total antioxidant capacity in follicular fluid Total antioxidant capacity (TAC) in follicular fluid was measured using oxygen radical absorbance capacity (ORAC) adapted for semi-automated measurement on a 96-well microplate reader (Synergy HT; BioTek, Winooski, VT) [13].

CoQ10 content and oxidative status in follicular fluid CoQ10 content in follicular fluids was assayed by high-performance liquid chromatography (HPLC) with electro-chemical detector (ECD) by Shiseido Co. Ltd., auto-sampler Model 3033,

Table 1 Characteristics of normal-weight, overweight, and obese women included in the study

	Normal−weight (BMI ≤ 24.9 kg/m^2) (n = 38)	Overweight (25 kg/m^2 ≤ BMI ≤ 29.9 kg/m^2) (n = 12)	Obese (BMI ≥ 30 kg/m^2) (n = 8)	Overweight/obese (BMI ≥ 25 kg/m^2) (n = 20)
AGE (years)	37.1 ± 0.8	35.8 ± 1.5	35.9 ± 2.3	36.0 ± 1.2
BMI (kg/m^2)	22.1 ± 0.2	27.1 ± 0.4*	32.4 ± 1.1*#	29.1 ± 0.72*

*$p < 0.001$ vs normal-weight women

#$p < 0.001$ vs overweight women

Data are expressed as mean ± SE

switch valve Model 3012, concentration column CQC and separation column CQS, and pump one and two Model 3001 (Shiseido Co. Ltd., Japan). CoQ10 levels were quantified following a single dilution step after extraction of 50 μl of FF with 250 μl propanol by vigorously mixing on a vortex as described by Silvestri et al. [27]

Lipid hydroperoxides in follicular fluids The levels of lipid hydroperoxides were determined in FF samples using ferrous oxidation-xylenol orange assay as previously described in plasma samples [1]. The levels of lipid hydroperoxides were quantified using a stock solution of t-butyl hydroperoxide. Lipid hydroperoxides are reported as μmol/l of FF.

PON1 activities in follicular fluids

PON1 activities were assayed in FF by using three substrates: phenyl acetate, paraoxon, or dihydrocoumarin. All assays of PON1 activities were performed in a 96-well plate, in a total reaction volume of 200 μl. Each 96-well plate included blank samples to monitor spontaneous hydrolysis of substrates [28].

Paraoxonase activity Ten microliters of FF was used. The basal assay mixture included 5 mmol/l Tris-HCl, pH 7.4 containing 1 mmol/l $CaCl_2$, and 1.0 mmol/l paraoxon. Paraoxon hydrolysis was spectrophotometrically monitored for 8 min (every 15 s) at 412 nm. One unit of PON1 paraoxonase activity was equivalent to 1 nmol of paraoxon hydrolyzed/min/ml.

Arylesterase activity FF samples were diluted 1:10 with 1 mmol/l $CaCl_2$ in 50 mmol/l Tris-HCl, pH 8.0, and then, 5 μl was taken for a total reaction volume of 200 μl. After addition of the substrate phenyl acetate (1 mmol/l), the hydrolysis was monitored at 270 nm for 3 min (every 15 s). One unit of arylesterase activity was equivalent to 1 μmol of phenyl acetate hydrolyzed/min/ml.

Lactonase activity FF samples were diluted 1:10 with 1 mmol/l $CaCl_2$ in 50 mmol/l Tris-HCl, pH 8.0, and 3 μl was then taken for the assay. After addition of the substrate dihydrocoumarin (DHC) (1.0 mmol/l), the hydrolysis was monitored at 270 nm for 10 min (every 15 s). One unit of lactonase activity was equivalent to 1 μmol of DHC hydrolyzed/min/ml.

DHR-based cell-free assay of HDL functions

HDL function was assessed using a fluorometric biochemical cell-free assay based on oxidation of the fluorogenic probe dihydrorhodamine 123 (DHR) [28, 29]. This assay assesses the intrinsic ability of HDL to be oxidized by measuring increasing fluorescence due to DHR oxidation over time [29]. A high oxidation rate of DHR indicates a high intrinsic ability of HDL to be oxidized. Several studies have used DHR-based cell-free assay to investigate functional properties of plasma HDL [29, 30]; we used the assay in FF for the first time.

In details, FF-HDL were prepared by selective precipitation with polyethylene glycol. A stock solution of DHR (50 mM) was diluted 1:1,000 in iron-free *N*-2-hydroxyethylpiperazine-*N*-2-ethanesulfonic acid-buffered saline (HBS; HEPES 20 mM, NaCl 150 mM, pH 7.4) prepared as previously described [29]. In a 96-well plate, aliquots of FF-HDL (1.25 μg HDL-cholesterol) and DHR working solution (final concentration 7 μM) were added, and the volume was diluted to 200 μl with HBS buffer. Immediately following DHR addition, the plate was protected from light and placed in a fluorescence plate reader. The fluorescence of each well was assessed at 2-min intervals for 1 h with a Synergy 2 multimode microplate reader (Biotek, VT) using a 485/538-nm excitation/emission filter. The oxidation rate was calculated for each well as the slope for the linear regression of fluorescence intensity between 10 and 50 min. DHR oxidation rate (DOR) was expressed as fluorescence units per minute (FU/min). The value was calculated as the mean of quadruplicates for the wells containing only DHR and for samples containing DHR and individual samples.

Statistical analysis

Results were expressed as mean ± standard error (SE). Differences between groups were determined using Student's t test. A p value < 0.05 was accepted as statistically

significant. Pearson's correlation coefficient and p value were used to show correlations and their significance; $p \leq 0.05$ is considered statistically significant (Software Microcal Origin 5.0, OriginLab).

Results

Fifty-eight women were included in the study; 65.5% of women had a normal body weight and 34.5% were overweight or obese; in details, 20.7% was overweight and 13.8% was obese. Table 2 summarizes the levels of HDL-C, triglycerides, and total protein content in FF in normal-weight and overweight or obese women. No significant modifications were observed in the levels of lipids and protein content in FF from the normal and overweight or obese subjects.

Lipid peroxidation and antioxidant capacity in FF

The levels of lipid hydroperoxides in FF of normal-weight women ranged from 0.12 to 5.64 μmol/l. As reported in Table 2, a higher level of lipid hydroperoxides was observed in FF obtained from overweight or obese women with respect to normal-weight women. Obese women showed higher levels of FF lipid hydroperoxides, but the differences were not statistically significant (Table 2).

The total antioxidant capacity in FF, evaluated using ORAC assay, ranged from 2.5 to 10.6 mmol TE/l in FF of normal-weight women. As reported in Table 2, the mean value of the antioxidant capacity (TAC) observed in FF of overweight or obese women was significantly lower compared with that in FF of normal-weight women. Significantly lower TAC values were observed in FF from obese women compared with overweight women (Table 2).

Levels of coenzyme Q10 were higher in FF from overweight/obese women (Table 2). This difference was maintained even after normalization by HDL content reaching higher levels of significance. Moreover, percentage of oxidized CoQ was significantly lower in overweight/obese patients (Table 2).

Antioxidant properties of FF-HDL

As reported in Fig. 1, the mean values of oxidation rate of DHR (DOR) in FF-HDL from overweight or obese women were significantly higher with respect to FF-HDL from normal-weight subjects. FF-HDL from obese women showed higher oxidation rate of DHR, but the differences were not statistically significant (Fig. 1). These results demonstrate that FF-HDL from overweight and obese women have a higher intrinsic ability to be oxidized.

PON1 activities (paraoxonase, arylesterase, and lactonase) are summarized in Table 3. The mean values of the PON1 activities were significantly lower in FF from overweight/obese compared with that from normal-weight women. As reported in Table 3, the modification of PON1 activities was realized at higher extent in FF from obese women compared to overweight women. PON1 is associated with FF-HDL; therefore, the normalized enzyme activity value for HDL-C in FF (PON1/HDL-C) was calculated. As summarized in Fig. 2, there were also significant differences in the ratio of PON1/HDL-C in FF from normal-weight and overweight or obese subjects.

Correlations

A significant negative correlation was established between PON1 paraoxonase activity and levels of lipid peroxides in

Table 2 Composition of follicular fluid of normal-weight, overweight, and obese women

	Normal weight (BMI ≤ 24.9 kg/m^2) (n = 38)	Overweight (25 kg/m^2 ≤ BMI ≤ 29.9 kg/m^2) (n = 12)	Obese (BMI ≥ 30 kg/m^2) (n = 8)	Overweight/obese (BMI ≥ 25 kg/m^2) (n = 20)
Protein (mg/ml)	58.3 ± 1.9	58.3 ± 2.0	65.2 ± 4.5	59.7 ± 3.9
HDL-C (mg/dl)	24.7 ± 0.9	23.2 ± 1.0	23.4 ± 1.8	23.2 ± 0.9
TG (mg/dl)	13.7 ± 0.7	16.9 ± 2.7	16.9 ± 3.3	16.2 ± 2.1
Lipid peroxides (μmol/l)	1.16 ± 0.11	1.84 ± 0.28*	2.49 ± 0.75*	2.10 ± 0.33*
Total antioxidant capacity (mmol TE/l)	5.96 ± 0.15	4.72 ± 0.41*	3.95 ± 0.42*$^{\#}$	4.37 ± 0.36*
CoQ10 (nmol/l): normalized CoQ10/HDL-C (nmol/mmol)	46.4 ± 3.5; 71.5 ± 4.1	74.0 ± 15.6*; 115.5 ± 22.9*	64.3 ± 8.1*; 98.8 ± 12.8*	69.9 ± 12.2*; 111.6 ± 15.6*
oxCoQ10/total CoQ10 (%)	24 ± 1	18 ± 0.4*	16 ± 0.5*	18 ± 0.4*

*$p < 0.05$ vs normal-weight women

$^{\#}$ $p < 0.05$ vs overweight women

Data are expressed as mean ± SE

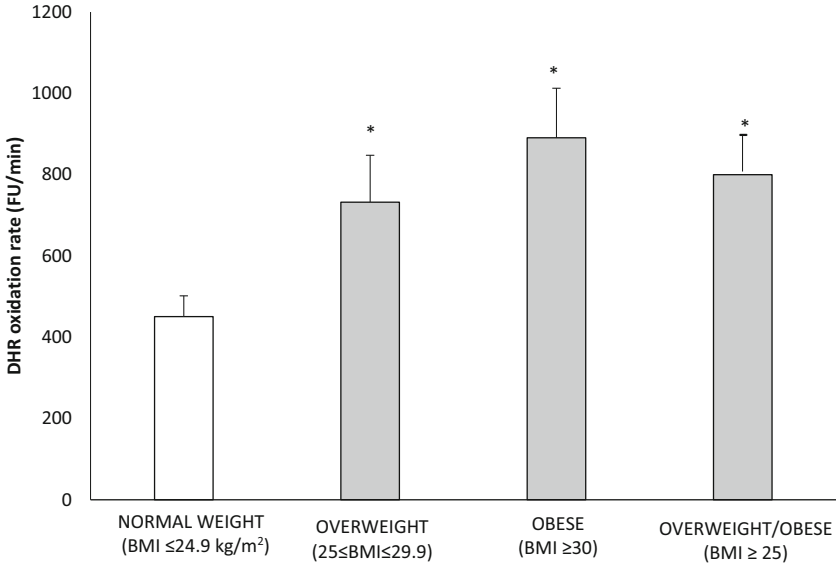

Fig. 1 Oxidation rate of DHR (DOR) in FF-HDL from normal weight, overweight and obese women. Data are reported as mean ± SE. *$p < 0.001$ vs normal weight women

FF ($r = -0.33$; $n = 58$; $p < 0.01$). The DHR oxidation rate (DOR) values of FF-HDL were negatively correlated to PON1 activities ($r = -0.63$; $n = 58$; $p < 0.001$) and total antioxidant capacity in FF ($r = -0.62$; $n = 58$; $p < 0.001$). Furthermore, DOR values were positively correlated to lipid peroxide levels in FF ($r = 0.45$; $n = 58$; $p < 0.001$). In addition, DOR values were positively associated with BMI ($r = 0.42$; $n = 58$; $p < 001$).

We investigated the relationship between FF-HDL levels and functional activity (PON1 activity and DHR oxidation rate values) and clinical markers for oocyte and embryo quality. As summarized in Table 4, a significant correlation was observed between PON1 activity in FF and DOR values of FF-HDL versus the number of oocytes retrieved or the number of "good quality oocytes." No significant correlations were observed between FF-HDL functional properties and clinical markers for embryo quality (Table 4). No significant correlations were observed between HDL-C levels and clinical parameters of oocyte and embryo quality.

Discussion

The relationship between obesity and infertility is well known along with the association between obesity and systemic oxidative stress [1–5]. Our study confirmed that obesity is associated with an increased oxidative stress as demonstrated by the higher levels of lipid hydroperoxides in FF of overweight and obese women compared with normal-weight women. Moreover, lower PON1 activities have been shown in FF from these patients in the absence of modifications of HDL-C levels of FF in overweight/obese and normal-weight women. Using a fluorometric biochemical cell-free assay, our data demonstrated that FF-HDL obtained from overweight/obese women are characterized by higher DHR oxidation rate (DOR) values indicating a higher intrinsic ability to be oxidized compared with FF-HDL from normal-weight women. A significant positive correlation has been observed between DOR values and BMI. Previous studies carried out in human plasma HDL suggested that DHR oxidation rate may represent a measure of

Table 3 Paraoxonase-1 activities in follicular fluid (FF) obtained from normal-weight, overweight, and obese women

	Nomral−weight (BMI ≤ 24.9 kg/m²) ($n = 38$)	Overweight (25 kg/m² ≤ BMI ≤ 29.9 kg/m²) ($n = 12$)	Obese (BMI ≥ 30 kg/m²) ($n = 8$)	Overweight/obese (BMI ≥ 25 kg/m²) ($n = 20$)
FF PON1 activities				
Paraoxonase (U/ml)	62.2 ± 5.6	40.7 ± 9.5*	25.5 ± 5.3*	34.5 ± 5.7*
Arylesterase (U/ml)	52.4 ± 3.2	40.3 ± 3.7*	30.7 ± 2.8*#	35.9 ± 2.8*
Lactonase (U/ml)	6.9 ± 0.3	5.9 ± 0.4*	4.6 ± 0.5*#	5.3 ± 0.4*

*$p < 0.001$ vs normal-weight women

# $p < 0.001$ vs overweight women

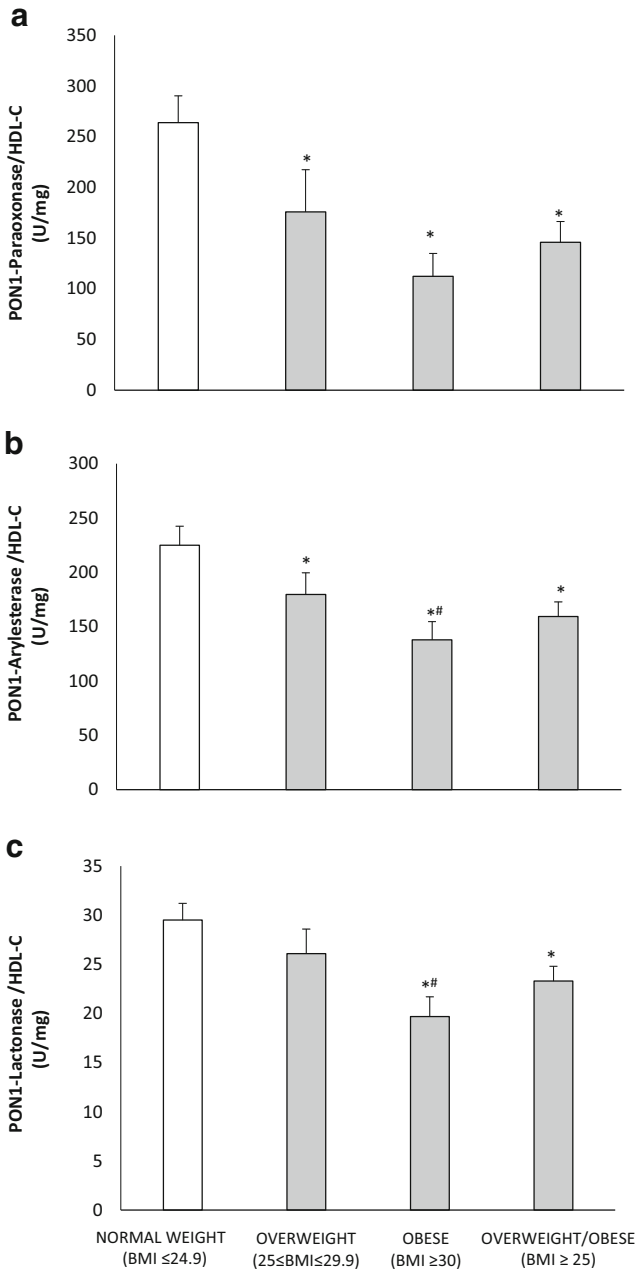

Fig. 2 Normalized paraoxonase (**a**), arylesterase (**b**) and lactonase (**c**) activities of PON1 in FF from normal-weight, overweight and obese women. *$p < 0.05$ vs normal weight, #$p < 0.05$ vs overweight women

alterations of HDL functionality; in fact, a higher DOR correlates significantly with proinflammatory properties of HDL and impaired ability of HDL to prevent lipid peroxidation [29, 30]. Therefore, we hypothesize that FF-HDL of obese women are more sensitive to lipid peroxidation and/or show dysfunctional properties. Previous data suggested that HDL-associated PON1 contributes to the antioxidant activity of plasma HDL and plasma HDL susceptibility to lipid peroxidation [1–3, 31–33]. In good agreement, we observed that PON1 activity in FF was negatively correlated with the

Table 4. Correlation between FF-HDL functional parameters (PON1 paraoxonase activity and DHR oxidation rate) and clinical parameters of oocyte and embryo quality. Table shows Pearson correlation coefficients and significance *$p<0.05$

CLINICAL PARAMETERS OF OOCYTE AND EMBRYO QUALITY	FF –HDL FUNCTIONAL PARAMETERS	
	PON1 - activity	DHR oxidation rate
number of oocytes retrieved	0.48*	-0.41*
number of "good quality oocytes"	0.54*	-0.52*
fertilization rate	0.14	0.18
percentage of embryos (number of embryos/number of oocytes)	0.06	0.17
number of embryos transferred	0.28	-0.21

variation of DHR oxidation ability of FF-HDL and total antioxidant capacity in FF confirming that PON1 could contribute to the antioxidant role of HDL in FF. To the best of our knowledge, this is the first study that investigates FF-HDL functionality and the relationship with PON1 activity in FF.

In an attempt to describe the relevance of HDL-associated antioxidant defenses to follicular maturation process, we have also investigated the content and oxidative status of CoQ10 in follicular fluids. CoQ10 has been reported in the human follicular fluid [22] and its content has been related to optimal embryo morphokinetic parameters and pregnancy rates [34]. Moreover, it was also reported that the age-related decline in oocyte quality and quantity could be reversed by the administration of CoQ10 [35]. Despite the well-known role of CoQ10 in lipoproteins and its promising effect in female reproduction, in our experimental setting, we did observe that CoQ10 levels were not related to total antioxidant capacity and not involved in FF-HDL intrinsic ability to be oxidized. In fact, we observed higher levels of CoQ10 and a higher extent of CoQ10 oxidation in FF isolated from overweight/obese women compared with FF from normal-weight women. Such modulation of CoQ10 status might suggest an adaptive response activated in obese/overweight women to counteract an increased oxidative environment. In this case, the effect of such a strategy is however not sufficient to maintain an optimal redox status with clear negative functional consequences. Alternatively, since FF lipoproteins are derived from serum through a HDL-selective blood-follicle barrier [18], increased CoQ10 content might be also linked to an enhanced permeability of the barrier allowing filtration of significant amounts of CoQ10-rich LDL particles. In fact, LDL and ox-LDL have been observed in FF of obese women [36] and this might significantly alter the overall FF content since LDL CoQ10 content is significantly higher compared with HDL.

Serum filtration of lipoproteins in FF may also explain the mechanisms involved in the lower PON1 activities in obese and overweight women. In fact, our results may suggest that the lower FF-PON1 enzyme activities are related to the modifications of HDL in plasma of obese women [20]. Previous studies have shown that obesity is associated with alterations of HDL composition and functionality with higher levels of lipid peroxides and a lower ability to protect erythrocyte membranes against lipid peroxidation due to a lower PON1 activity [1–3].

Some hypotheses on the physio-pathological relevance of our results can be proposed. The effects of oxidative stress on female reproduction have been recently reviewed [37]. Previous studies have shown that elevated FF lipid peroxidation level may have a negative impact on in vitro fertilization outcome [38]. A role of PONs in follicle maturation has also been proposed with a positive correlation between PON1 arylesterase activity and paraoxonase activity and total number of retrieved oocytes [[21]39]. An association was reported by other authors between PON1 activity in follicular fluid and embryo morphology parameters' individual embryo cell number and individual embryo fragmentation score [40].

In our previous study, carried out in the same overweight/obese and normal-weight subjects undergoing ART procedures, we reported a lower number and quality of oocytes and embryos in overweight/obese compared with normal-weight women [16]. However, there were no statistically significant differences in pregnancy outcomes, most likely because of small patient group and because of the heterogeneity of factors that influence embryo-implantation and fetal development. In the present study, we observed an association between biochemical markers of HDL function (PON1 activity and FF-HDL oxidation rate) and clinical parameter of oocyte quality among women undergoing in vitro fertilization; while no significant correlation was observed with HDL cholesterol levels. Therefore, we could suggest that the impaired FF-HDL functionality observed in obese and overweight women could explain the lower number and quality of oocyte and embryo observed in these women undergoing ART procedures.

HDL exert pleiotropic roles in FF that include promotion of cholesterol efflux and the anti-inflammatory and antioxidative properties that are critically important in the health of the human oocyte. Our data suggest that the quality of FF-HDL, more than the levels of HDL-C in FF, is an important determinant for oocyte quality. Therefore, targeting FF-HDL functionality, in addition to FF-HDL-C levels, may represent a promising and interesting biomarker for reproductive outcomes. Studies looking into the specific mechanisms leading to a loss of FF-HDL functionality are of particular importance, in order to develop therapeutic approaches that aim to restore the HDL properties in FF.

Funding information The study was supported by grants from Department of Life and Environmental Sciences (to TB, LT) and Department of Clinical Sciences (to FG, A.V) of Polytechnic University of Marche. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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