The anti-inflammatory function of follicular fluid HDL and outcome of modified natural cycle-in vitro fertilization

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Published in:
Biology of Reproduction

DOI:
10.1093/biolre/ioaa061

Publication date:
2020

Biology of Reproduction, 2020, 103(1), 7–9
doi:10.1093/biolre/ioaa061
Letter to the Editor
Advance Access Publication Date: 25 April 2020

Letter to the Editor

The anti-inflammatory function of follicular fluid HDL and outcome of modified natural cycle in vitro fertilization†

Dear Editor,

Cholesterol homeostasis plays an important role in oocyte development and fertility [1]. In follicular fluid (FF), the environment surrounding the developing oocyte, high-density lipoproteins (HDLs) are the predominant carriers of cholesterol [2, 3]. Within the cardiovascular field, the focus of research is currently changing from measuring the static biomarker HDL cholesterol (HDL-C) toward determining the functional properties of HDL particles [4]. With respect to the field of reproductive medicine, an interesting recent study in mice showed that a decreased cholesterol efflux function of FF-HDL results in impaired fertility [5]. However, next to promoting cholesterol efflux, HDL particles also have potent anti-inflammatory properties [4, 6]. Accumulating evidence indicates that inflammatory disorders alter the composition of FF and result in infertility due to reduced oocyte quality [7, 8]. Conceivably, HDL present in FF might modulate the local inflammatory state within the follicle. However, currently, to the best of our knowledge, no data exist exploring such a hypothesis either in humans or preclinical models. Therefore, the present study was designed (i) to characterize anti-inflammatory properties of FF-HDL in relation to systemic HDL and (ii) to determine if the anti-inflammatory function of FF-HDL is associated with the outcomes of modified natural cycle in vitro fertilization (MNC-IVF), a procedure close to normal human reproductive physiology [9].

The study included 326 MNC-IVF cycles from 198 patients collected during either routine medical care (August 2013–July 2014) or an observational cohort study to relate nutrition, biomarkers and MNC-IVF outcomes (October 2014–March 2018, The Netherlands Trial Register number NTR4409). For further details on cohort and MNC-IVF procedure, please see [9] and Supplementary Methods. Inclusion criteria were: growth of a single dominant follicle; retrieval of one oocyte; minimal macroscopic blood contamination; and maximum cycle number of six. Medical ethics committee approval was requested, but waived, since FF is considered waste material. Patients consented to blood draws. Top quality embryos were defined as two pronuclei on day 1 and four cells, no multinucleated blastomeres, and less than 20% fragmentation on day 2. Single embryo transfer took place on day 2, and the occurrence of pregnancy was confirmed by a positive serum HCG test 2 weeks later. The anti-inflammatory capacity of HDL was assessed as the ability to inhibit tumor necrosis factor α-induced VCAM-1 mRNA expression in endothelial cells in vitro (higher values indicate higher anti-inflammatory capacity), as previously published [6] using 2% (v/v) individual apoB-depleted plasma or FF samples or phosphate buffered saline (Online Supplement). Results are expressed as median [interquartile range]. Wilcoxon Signed Ranks test, Spearman correlations, and multilevel analysis using generalized estimating equations (GEEs) were applied as appropriate (SPSS 23) (Supplementary Methods). A P-value <0.05 was considered statistically significant.

Median age throughout all cycles was 31.9 [29.3–33.8] years and BMI 23.3 [21.1–26.1] kg/m^2 (27% overweight, 4.9% obese). Prior to IVF treatment, in 50.3% of cycles alcohol was consumed regularly and in 9.8% the patient was a smoker. Median subfertility duration was 35.6 [22.3–50.4] months. In 19 randomly selected first cycle MNC-IVF patients, FF-HDL anti-inflammatory capacity was comparable to that of matched plasma HDL (FF: 13.8% [3.4–24], plasma: 17.4% [8.9–25], $P = 0.872$). However, no significant correlation between the anti-inflammatory capacity of the two matrices was found ($r = 0.098$, $P = 0.689$). To explore the contribution of FF-HDL to the whole FF anti-inflammatory capacity, eight patients undergoing first cycle MNC-IVF were randomly selected and only for this experiment HDL was isolated by fast protein liquid chromatography. Compared with whole FF, FF-HDL had a significantly higher, although quantitatively moderate, anti-inflammatory capacity (15% [12–19] versus 20% [16–23] reduction in VCAM-1 mRNA related to the full TNF-α-induced induction, $P = 0.012$) indicating that HDL might be a physiologically relevant contributing factor to the protective function of FF against inflammation. In all 326 MNC-IVF cycles, a higher FF-HDL anti-inflammatory function was related to an increased chance of the oocyte to develop into a top-quality embryo in unadjusted analyses (Table 1). This result remained significant also after adjustment for age, BMI, smoking, alcohol, and fertilization method (odds ratio per % increase in anti-inflammatory function: 1.02 [95% confidence interval: 1.00–1.03], per standard deviation (16.62%) increase in anti-inflammatory function: 1.33 [1.06–1.68], $P = 0.016$; Table 1). No significant relationship with the occurrence of pregnancy was found.

Taken together, the results of the present study indicate that, in addition to a role in cholesterol transport, FF-HDL have anti-inflammatory properties that, at least under the assay conditions used in the present work, positively associate with certain early developmental parameters of the oocyte. FF-HDL is considered to be largely derived from plasma [1, 2, 5]; however, they differ in size and composition compared with plasma HDL by containing less cholesterol and more phospholipids [2]. In our study, the lack of a relationship between the anti-inflammatory capacities of FF and matched plasma HDL indicates that FF may contain anti-/pro-inflammatory factors specific to the ovarian environment. It is unclear, at present, if the differences in anti-inflammatory function between the matrices are due to remodeling occurring during the passage of plasma HDL into the follicle or if anti-/pro-inflammatory

Table 1. GEEs analysis of the relationship between embryo development in modified natural cycle-IVF/ICSI and FF anti-inflammatory capacity.

		FF anti-inflammatory capacity (%)	Unadjusted model		Adjusted model	
			Odds ratio [95% CI] upper: per 1% increase lower: per SD increase	*P* value	Odds ratio [95% CI] upper: per 1% increase lower: per SD increase	*P* value
Top quality embryo	Yes ($n = 106$)	15.28 [5.38–26.04]	**1.01 [1.00–1.03]**	**0.031**	**1.02 [1.00–1.03]**	**0.016**
	No ($n = 199$)	12.36 [0.14–24.64]	**1.26 [1.02–1.55]**		**1.33 [1.06–1.68]**	
Pregnancy	Yes ($n = 55$)	13.53 [4.33–30.14]	1.00 [0.99–1.02]	0.741	1.01 [0.99–1.03]	0.342
	No ($n = 161$)	13.60 [4.83–24.60]	1.05 [0.77–1.44]		1.17 [0.85–1.62]	

Adjusted models include: age, BMI, smoking, alcohol, and method (for top quality embryo); and smoking, alcohol and duration of subfertility (for pregnancy). One SD equals 16.62% anti-inflammatory capacity. Bold values: $P < 0.05$. CI, confidence interval. SD, standard deviation.

factors are produced locally by granulosa cells, theca cells, or the oocyte, which then associate with FF-HDL particles. Important advantages of our study are the prospective design and the use of MNC-IVF, which is closer to normal physiology compared with classical hyperstimulation IVF and allows for an individual correlation of FF composition with single oocyte and embryo characteristics. Potential limitations are that patients were from a single center and that only one selected anti-inflammatory function was tested and not complete pro-/anti-inflammatory profiles generated. However, the current study indicates that such studies would be worthwhile to be carried out. Furthermore, no relationship between FF-HDL anti-inflammatory capacity and pregnancy was found, likely due to the fact that pregnancy is the result of multiple factors, including endometrial and spermatozoa function. Additional research is necessary to explore the mechanism behind the FF-HDL anti-inflammatory capacity (e.g., quantify FF-HDL components that exert anti-inflammatory function), since such an approach offers the potential to identify not only clinically relevant biomarkers for natural and assisted reproduction but also potential targets for therapeutic intervention.

Supplementary data

Supplementary data is available at *BIOLRE* online.

Conflict of interest

The Department of Obstetrics and Gynecology received an unrestricted educational grant from Ferring Pharmaceutical Company, The Netherlands. The other authors have declared that no conflict of interest exists.

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†**Grant Support:** CJ was supported by a fellowship from the Chinese Scholarship Council.

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References

1. van Montfoort AP, Plosch T, Hoek A, Tietge UJ. Impact of maternal cholesterol metabolism on ovarian follicle development and fertility. *J Reprod Immunol* 2014; **104–105**:32–36.
2. Jaspard B, Fournier N, Vieitez G, Atger V, Barbaras R, Vieu C, Manent J, Chap H, Perret B, Collet X. Structural and functional comparison of HDL from homologous human plasma and follicular fluid. A model for extravascular fluid. *Arterioscler Thromb Vasc Biol* 1997; **17**: 1605–1613.
3. Gautier T, Becker S, Drouineaud V, Menetrier F, Sagot P, Nofer JR, von Otte S, Lagrost L, Masson D, Tietge UJ. Human luteinized granulosa cells secrete apoB100-containing lipoproteins. *J Lipid Res* 2010; **51**: 2245–2252.
4. Triolo M, Annema W, Dullaart RP, Tietge UJ. Assessing the functional properties of high-density lipoproteins: an emerging concept in cardiovascular research. *Biomark Med* 2013; **7**:457–472.
5. Quiroz A, Molina P, Santander N, Gallardo D, Rigotti A, Busso D. Ovarian cholesterol efflux: ATP-binding cassette transporters and follicular fluid HDL regulate cholesterol content in mouse oocytes. *Biol Reprod* 2020; **102**:348–361.
6. Ebtehaj S, Gruppen EG, Parvizi M, Tietge UJF, Dullaart RPF. The anti-inflammatory function of HDL is impaired in type 2 diabetes: role of hyperglycemia, paraoxonase-1 and low grade inflammation. *Cardiovasc Diabetol* 2017; **16**:132.
7. Snider AP, Wood JR. Obesity induces ovarian inflammation and reduces oocyte quality. *Reproduction* 2019; **158**:R79–R90.

8. Li Y, Li R, Ouyang N, Dai K, Yuan P, Zheng L, Wang W. Investigating the impact of local inflammation on granulosa cells and follicular development in women with ovarian endometriosis. *Fertil Steril* 2019; **112**: 882–891.e1.

9. Nagy RA, van Montfoort AP, Dikkers A, van Echten-Arends J, Homminga I, Land JA, Hoek A, Tietge UJ. Presence of bile acids in human follicular fluid and their relation with embryo development in modified natural cycle IVF. *Hum Reprod* 2015; **30**:1102–1109.