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Endothelial function and concentrations of high-sensitivity C-reactive protein, interleukin-6, and tumor necrosis factor-alpha during a long agonist IVF protocol

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ABSTRACT

We examined possible changes in endothelial function during a long agonist *in vitro* fertilization (IVF) protocol. We measured flow-mediated dilatation (FMD) and FMD percent (FMD%) from the brachial artery and plasma levels of high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-⟨). We studied longitudinally three time points in 27 women undergoing a long agonist IVF treatment at Kuopio University Hospital. The first visit was at the beginning of their period (low estradiol). The other two visits were during gonadotrophin-releasing hormone (GnRH) analog downregulation (low estradiol) and at the end of follicle-stimulating hormone (FSH) stimulation (high estradiol). The first visit was used as the reference, and the women served as their own controls. During the stimulation protocol, FMD and FMD% remained. Toward the end of stimulation, hsCRP ($P = 0.003$), IL-6 ($P = 0.04$), and TNF-⟨ ($P = 0.008$) concentrations all decreased, while estradiol levels increased ($P < 0.001$). Correlations between estradiol and proinflammatory factors or FMD were, however, non-significant. The only significant correlation appeared between FMD% and hsCRP at Visit 2 ($r = 0.485$, $P = 0.01$). In conclusion, IVF stimulation promoted no change in endothelial function, whereas hsCRP, IL-6, and TNF-⟨ decreased. These findings indicate that estrogen may improve the cytokine profile among healthy women undergoing IVF, but this is not reflected in endothelial function.

1. Introduction

Estradiol has a beneficial impact on endothelial function. It improves vasodilatation and prevents blood vessel injury and the development of atherosclerosis (Farhat et al., 1996; Mendelsohn, 2002; Tostes et al., 2003). One third of the protective effects of estradiol is due to the changes in the lipid profile, and the rest of it comes from direct actions on the arterial wall (Mendelsohn, 2002). Endothelial function regulates arterial health, and its dysfunction may lead to cardiovascular morbidity and death (Gokce et al., 2003; Raitakari, Celermajer, 2000; Schächinger et al., 2000).

Endothelial function can be examined by measuring brachial artery flow-mediated dilatation (FMD). Endothelial cells respond to increased blood flow and shear stress, which causes vasodilators, especially nitric oxide release from those cells, resulting in arterial dilatation (Mullen et al., 2001). Therefore, measuring arterial diameter during

Abbreviations: FMD, Flow-mediated dilatation; FMD%, Flow-mediated dilatation percent; TNF-α, Tumor necrosis factor alpha; IL-6, Interleukin 6; hsCRP, high-sensitivity C-reactive protein; IV, F*in vitro* fertilization; FSH, Follicle stimulating hormone; GnRH, Gonadotrophin-releasing hormone; BMI, Body mass index; NS, non-significant; LDL, Low-density lipoprotein; HDL, High-density lipoprotein; SD, Standard deviation; SEM, Standard error of mean.

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postischemic reactive hyperemia provides valuable information about endothelial function. FMD% reflects the increase in arterial diameter compared to the baseline artery diameter. Endothelial function can also be examined by determining proinflammatory factors, because local inflammation has a significant role in developing atherosclerosis (Hansson, 2005; Libby, 2012). Proinflammatory factors, like hsCRP, IL-6, and TNF-⟨, down-regulate nitric oxide synthase, leading to vasoconstriction (Pearson et al., 2003), but they may also cause thrombocyte aggregation and thrombosis (Joseph et al., 2002).

Estradiol levels vary during the menstrual cycle, and studies about FMD are discordant. Williams et al. (2001) showed that endothelial function increased during the follicular phase, decreased after ovulation, and increased again during the luteal phase (Williams et al., 2001). However, two other studies found no significant differences in FMD during the menstrual cycle (D'Urzo et al., 2018) (Saxena et al., 2012). IVF cycles allow good opportunities to examine low and exceptionally high estradiol levels longitudinally in the same set of healthy female subjects. Long agonist IVF protocol uses a gonadotrophin-releasing hormone (GnRH) analog in the beginning of the treatment, suppressing hormone production from the ovaries and inducing postmenopausal-like low estradiol levels. FSH stimulation is then initiated, and estradiol levels rise considerably above unstimulated cycles. Approximately 30–50 % of the IVF patients in Kuopio University Hospital have been treated with long agonist stimulation since it allows flexible timing and often gives a stable number of equal-sized follicles (Lambalk et al., 2017;Pacchiarotti et al., 2016).

We have found previously that during a long agonist IVF protocol, the carotid artery stiffened during high estradiol levels compared to low or normal levels of estradiol. This phenomenon was not explained by the wider diameter of the carotid artery, hyperlipidemia, or blood pressure profiles (Leppänen et al., 2020). To better understand this phenomenon, we now evaluated whether IVF affects FMD and cytokine profiles in changing estrogen milieu. As far as we know, such a study setting has not been used in earlier studies.

2. Material and methods

2.1. Participants

The study protocol was approved by the Ethics Committee of the Northern Savo Hospital District. Written informed consents were obtained on forms from 27 infertile women, ranging from 24 to 40 years of age (33+/−1 years, mean+/−SD), and were approved by the same board before study participation.

Women were recruited from the Kuopio University Hospital Infertility Clinic while they visited the doctor, and IVF treatment was planned with a long agonist protocol. The study population was gathered during the years 2012–2016.

2.2. Study protocol

The women were examined three times. The first visit (Visit 1) was at the beginning of their menstrual cycle (two to five days after menstruation). Agonist medication (a GnRH analog, nafarelin acetate 800 mcg/day) was started one week before the next period. The following control was approximately one month later when patients had been using GnRH-analogue for approximately two weeks. At the second visit (Visit 2), the estradiol levels were supposed to be very low, and the women started controlled ovarian stimulation with FSH. The dose was adapted to the patient's body mass index (BMI), age, and antral follicle count, and 125–300 IU doses were used. Approximately ten days later, women met the doctor for the third time (Visit 3) after administering FSH daily, and their estradiol levels were expected to be increased. Blood samples were acquired, and clinical measurements were made at all three visits. For the hormonal intervention, primary outcome variables were FMD and flow-mediated dilatation percent (FMD%). Secondary outcome variables were the serum levels of hsCRP, IL-6, and TNF-⟨.

Height and weight were determined, and BMI was calculated. The weight was measured by a bioelectronic impedance analyzer (InBody 3.0; BioSpace, Seoul, Korea). Blood pressure was measured two times with an Omron M4i device (Matsusaka, Japan), and the results were averaged. All lipid and estradiol determinations were done using standard methods, as previously described (Leppänen et al., 2020). HsCRP was assessed with a Cobas 8000 (c502) analyzer (Hitachi High Technology Co, Tokyo, Japan) using C-Reactive Protein (Latex) High Sensitive Assay (Roche Diagnostics GmbH, Mannheim, Germany), with a working range of 0.15–20 mg/l. IL-6 was analyzed with R&D systems (a Bio-techne brand, HS600C, Quantikine HS ELISA high sensitivity, Catalog no. HS600C, LOT: P203572, Exp. 11 Sep 2019), with a working range 0.156 pg/mL–10 pg/mL. The TNF-⟨ was determined with R&D systems (a Bio-techne brand, HSTA00E, Quantikine HS ELISA high sensitivity. Catalog no. HSTA00E, LOT: P202744, Exp. 25 Oct 2019), with a working range 0.156 pg/mL– 10 pg/mL.

2.3. Ultrasound imaging

Ultrasound studies were performed using Sequoia 512 ultrasound mainframes (Acuson). Brachial FMD was assessed by measuring the diameter of the left brachial artery both at rest and after reactive hyperemia. The increased flow was induced by inflation of a pneumatic tourniquet placed around the forearm to a pressure of 250 mmHg for 4.5 min, followed by release. Three arterial diameter measurements were taken at end-diastole at a fixed distance from an anatomic marker at rest and 40, 60, and 80 s after cuff release. The FMD was expressed as the change in absolute diameter after reactive hyperemia and the percent change relative to the resting scan (FMD%)(Corretti et al., 2002).

2.4. Statistical analyses

Statistical analyses and calculations were done with IBM SPSS Statistics (version 25 for Macintosh, Armonk, New York). Descriptive data are expressed as means±SD in tables and as means±SEM in figures. The Kolmogorov–Smirnov test showed skewed distributions for most parameters. Therefore, Friedman's test, which is a non-parametric test, was used to assess the difference of the measurements among the visits. If the significance was found between three visits, then Wilcoxon's test was used to analyze differences between two visits. Univariate correlations were assessed using Spearman correlation. $P < 0.05$ was considered statistically significant.

3. Results

The characteristics of the 27 study participants are presented in Table 1. Estradiol levels changed statistically significantly ($P < 0.001$) (Table 1). The mean values of FMD and FMD% at three visits are shown in Fig. 1. The absolute FMD unchanged at Visits 1, 2, and 3 (Fig. 1). The FMD% also unchanged during three visits (Fig. 1). HsCRP concentrations were at the highest level at Visit 1 and then decreased at Visit 2 ($P = 0.003$) and Visit 3 ($P = 0.02$) (Fig. 2). The IL-6 levels were highest in Visit 2 and then decreased in Visit 3 ($P = 0.04$) (Fig. 2). The concentrations of TNF-⟨ decreased a little during the stimulation, from Visit 1 to Visits 2 and 3 ($P = 0.008$) (Fig. 2).

Five of the 27 study participants had higher levels of hsCRP than the others (>10 mg/l). Four of them had decreasing levels of hsCRP, whereas one had a rising trend. Three of them had endometriosis, one had subclinical hypothyreosis, and one had an unknown reason for possible inflammation. Thus, when the hsCRP level is more than 10 mg/l, low-level inflammation without a known cause may exist.

The correlation matrix is shown in Table 2. The only significant correlation appeared between FMD% and hsCRP at Visit 2 ($r = 0.485$, $P = 0.01$). Otherwise, the correlations were insignificant.

Table 1
Clinical characteristics and responses to a long agonist IVF protocol. The values are means ± SD.

	Visit 1	Visit 2	Visit 3	*P*-value
Age (years)	33 ± 4	–	–	–
Gravity	0.4 ± 0.6	–	–	–
Para	0.2 ± 0.4	–	–	–
Height (cm)	167 ± 4	–	–	–
Weight (kg)	69.0 ± 14.1	–	–	–
Body mass index (kg/m^2)	24.6 ± 5.1	–	–	–
Systolic blood pressure (mmHg)	123 ± 9	123 ± 11#	119 ± 11	0.026
Diastolic blood pressure (mmHg)	75 ± 8	75 ± 10	73 ± 8	0.34
Total cholesterol (mmol/l)	4.7 ± 0.9⊗	4.9 ± 0.8°	4.4 ± 0.7	<0.001
LDL cholesterol (mmol/l)	2.8 ± 0.8*◊	2.9 ± 0.8°	2.5 ± 0.7	<0.001
HDL cholesterol (mmol/l)	1.7 ± 0.3	1.8 ± 0.3	1.8 ± 0.3	0.15
Triglycerides (mmol/l)	0.7 ± 0.2	0.7 ± 0.3	0.7 ± 0.3	0.94
Estradiol (nmol/l)	0.2 ± 0.1*◆	0.1 ± 0.1°	6.4 ± 6	<0.001

Significances: # $P < 0.05$ Visit 2 vs Visit 3, ⊗ $P < 0.01$ Visit 1 vs Visit 2, ° $P < 0.001$ Visit 2 vs Visit 3, *$P < 0.05$ Visit 1 vs Visit 2, ◊ $P < 0.01$ Visit 1 vs Visit 3, * $P < 0.001$ Visit 1 vs Visit 2, ◆ $P < 0.001$ Visit 1 vs Visit 3.

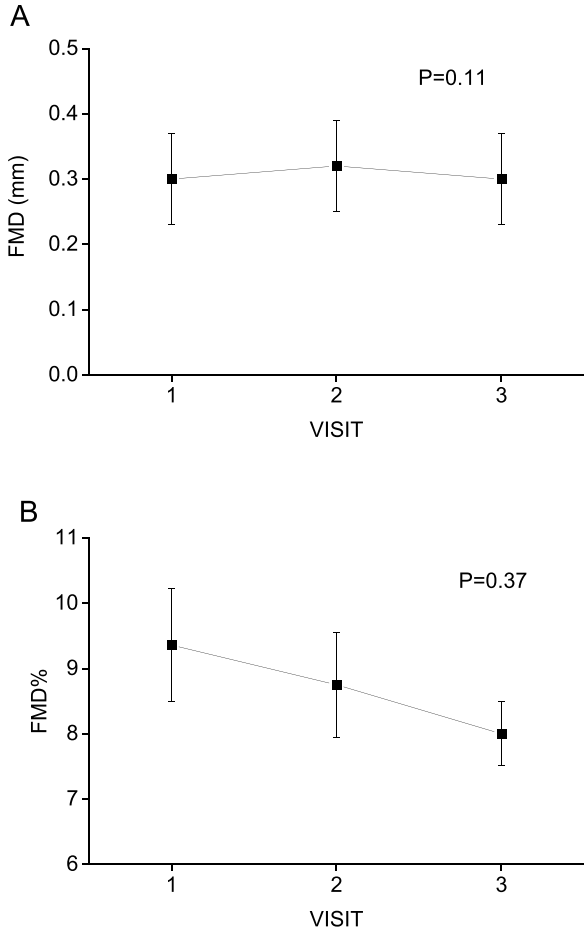

Fig. 1. The values of flow-mediated dilatation (FMD) and flow-mediated dilatation percent (FMD%) in visits 1, 2 and 3. The values are means ± SEM. Statistical analyses were done with Friedman's test.

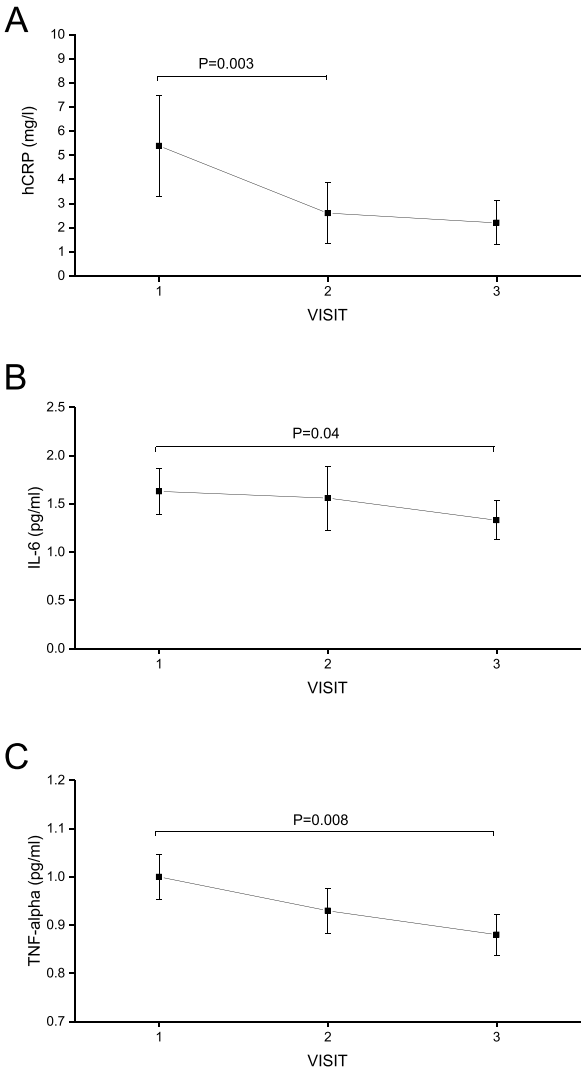

Fig. 2. The values of high-sensitivity C-reactive protein (hsCRP), interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) at Visits 1, 2 and 3. The values are means ± SEM. Statistical analysis were done with Wilcoxon's test and then with Friedman's test.

4. Discussion

We found that, during the stimulation phase of a long agonist IVF protocol, when the estradiol level increased, hsCRP, IL-6, and TNF-⟨ concentrations decreased. However, this was not reflected in endothelial function since FMD and FMD% were unchanged in IVF cycles. Thus, estrogen may improve the cytokine profile among healthy women undergoing IVF, but this is not reflected in endothelial function.

We had found earlier that, during a long agonist IVF protocol, the carotid artery stiffened during high estrogen levels, and this stiffening was not due to the wider diameter of the carotid artery, high blood pressure, or hyperlipidemia (Leppänen et al., 2020). This finding motivated us to study endothelial function during the stimulation protocol. However, no impact on endothelial function was found as measured by FMD and FMD%. Therefore, FMD changes fail to explain our earlier somewhat alarming findings on carotid artery distensibility during the same stimulation protocol. In contrast, toward the end of stimulation, when estradiol levels rose, proinflammatory factors, such as hsCRP, IL-6, and TNF-⟨, diminished. Our results also conflict with previous findings during long agonist IVF stimulation (Orvieto et al., 2004; Persson et al., 2012), in which controlled ovarian stimulation induced a state of systemic inflammation.

Table 2
Correlations between flow-mediated dilatation (FMD) or FMD percent (FMD%) and different variables at Visits 1, 2, and 3. Spearman correlation coefficient was used. Significances: * $P < 0.05$.

	FMD			FMD%		
Visit	1	2	3	1	2	3
	r	r	r	r	r	r
Estradiol (nmol/l)	0.043	0.214	0.138	−0.147	0.012	−0.292
hsCRP (mg/l)	−0.316	−0.194	0.015	0.176	0.485*	0.193
IL-6 (pg/mL)	−0.188	0.061	−0.034	0.273	0.287	0.176
TNF-α (pg/mL)	−0.030	−0.235	0.073	0.292	0.250	0.114
Total cholesterol (mmol/l)	0.182	−0.086	0.085	−0.077	0.160	−0.139
LDL (mmol/l)	0.193	−0.035	0.069	−0.160	0.174	−0.057
HDL (mmol/l)	0.123	0.008	0.188	−0.145	−0.243	−0.232
Triglycerides (mmol/l)	0.173	0.222	0.176	0.088	0.083	0.040
Age (years)	0.197	−0.059	0.203	0.115	−0.165	0.065
Height (cm)	0.017	−0.160	−0.018	0.296	0.287	0.176
Weight (kg)	0.162	0.198	0.036	0.082	0.315	0.044
Systolic blood pressures (mmHg)	0.009	0.088	−0.283	0.146	−0.035	0.051
Diastolic blood pressures (mmHg)	0.217	0.213	−0.244	0.024	0.023	−0.010
Carotid artery distensibility (%/10 mmHg)	−0.260	−0.181	−0.028	0.062	0.020	−0.262
Carotid artery diameter (mm)	0.154	0.346	−0.203	−0.228	0.069	0.280

FMD reflects the capability of endothelial cells to produce vasodilators, especially nitric oxide, in response to increased blood flow and shear stress (Mullen et al., 2001). Nitric oxide is the most crucial molecule regulating endothelial function, but also other dilatory molecules exist, most notably prostacyclin and endothelial-derived hyperpolarizing factor (Stoner et al., 2012). Thus, the temporal development of dilatation and reversion is likely to be linked with the action of vasoactive substances and their elimination. Many factors can affect FMD, like arterial hypertension, elevated glucose and cholesterol levels, obesity, and estradiol, but for example, catecholamines and the sympathetic nervous system impact the artery's diameter without directly affecting FMD (Moens et al., 2005). Along with the main outcome variables, we also tested associations between FMD or FMD% and lipids, age, height, weight, systolic blood pressures, diastolic blood pressures, carotid artery distensibility, and carotid artery diameter. These are potential confounding variables, and they might have an intermediating role in the endothelial function (Moens et al., 2005). In our study, systolic blood pressure, total cholesterol, and LDL cholesterol descended statistically significantly during the supraphysiological estradiol levels, but endothelial function was unchanged. These findings are advantageous considering cardiovascular health. Although many factors can affect arterial wall function, endothelial function was unaltered during the significant hormonal changes in our study. It has been documented in animal studies that estrogen-induced effects depend on the vascular bed (Tostes et al., 2003). Estrogen treatment increases aortic stiffness and improves endothelial function in the hindquarters but not in the carotid vascular bed (Tostes et al., 2003). This phenomenon may also explain our findings of unchanged FMD.

Estradiol affects the immune response in humans (Straub, 2007). Studies about hsCRP concentrations during the menstrual cycle are conflicting. Some studies reported similar findings as in our study, in which hsCRP levels were highest in the early follicular phase and then decreased during the late follicular phase (Blum et al., 2005; Puder et al., 2006). According to one encompassing review article, most of the studies on IL-6 and TNF-⟨ secretion found that higher estradiol levels inhibited IL-6 and TNF-⟨ secretion (Straub, 2007). Little is known about the interplay between CRP and FMD, because only a few studies in this area exist. It has been shown earlier that elevated CRP serum levels are associated with impaired systemic endothelial vasodilator function in acute coronary syndromes (Fichtlscherer, Zeiher, 2000;Fichtlscherer et al., 2000). Elevated CRP levels affect even the arteries of healthy children by disturbing the endothelial function (Järvisalo et al., 2002). However, it has been reported that despite the increase in serum hsCRP levels in normal pregnant women, FMD improved (Saarelainen et al., 2009). This finding differs from our results about FMD and hsCRP during stimulated high estradiol concentrations.

The validity of this study can be considered good since all these women underwent the same IVF protocol in one unit, and they served as their own controls. FMD and FMD% were measured by medical personnel who had no information on the IVF cycle phase or earlier measurement values. Laboratory tests were also blindly analyzed. Because all women underwent the same treatment protocol, the cause of underlying infertility may play no role in endothelial function. A limitation of this study is the small number of subjects. The selection of the patients and limited statistical power in the analyses may affect our results. The results may apply only to fertile-age women with normal healthy endothelial function. FMD is a non-invasive method and allows repeated measurements. Our sonographers are very skilled and well trained, and they produced very good-quality pictures, although FMD as a method is quite difficult.

5. Conclusions

We found that endothelial function exhibited no change during a long agonist IVF protocol, whereas estradiol levels increased. Furthermore, toward the end of stimulation, proinflammatory factors (hsCRP, IL-6, and TNF-⟨) decreased, whereas estradiol levels rose. Thus, a long agonist IVF protocol had a favorable anti-inflammatory effect in this fertile-age study population with a healthy endothelium. Altogether, these results are reassuring that IVF stimulation, despite marked estrogen changes, is safe in terms of endothelial function.

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Declaration of Competing Interest

The authors have no competing interests to declare.

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References

Blum, C.A., Muller, B., Huber, P., Kraenzlin, M., Schindler, C., De Geyter, C., 2005. Low-grade inflammation and estimates of insulin resistance during the menstrual cycle in lean and overweight women". J. Clin. Endocrinol. Metab. 90 (6), 3230–3235.
Corretti, M.C., Anderson, T.J., Benjamin, E.J., Celermajer, D., Charbonneau, F., Creager, M.A., 2002. Guidelines for the ultrasound assessment of endothelial-dependent flow-mediated vasodilation of the brachial artery: a report of the International Brachial Artery Reactivity Task Force". J. Am. Coll. Cardiol. 39 (2), 257–265.
D'Urzo, K.A., King, T.J., Williams, J.S., Silvester, M.D., Pyke, K.E., 2018. The impact of menstrual phase on brachial artery flow-mediated dilatation during handgrip exercise in healthy premenopausal women". Exp. Physiol. 103 (2), 291–302.
Farhat, M.Y., Lavigne, M.C., Ramwell, P.W., 1996. The vascular protective effects of estrogen". FASEB J. 10 (5), 615–624.
Fichtlscherer, S., Rosenberger, G., Walter, D.H., Breuer, S., Dimmeler, S., Zeiher, A.M., 2000. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease". Circulation 102 (9), 1000–1006.

Fichtlscherer, S., Zeiher, A.M., 2000. Endothelial dysfunction in acute coronary syndromes: association with elevated C-reactive protein levels". Ann. Med. 32 (8), 515–518.
Gokce, N., Keaney, J.F., Hunter, L.M., Watkins, M.T., Nedeljkovic, Z.S., Menzoian, J.O., et al., 2003. Predictive value of noninvasively determined endothelial dysfunction for long-term cardiovascular events in patients with peripheral vascular disease". J. Am. Coll. Cardiol. 41 (10), 1769–1775.
Hansson, G.K., 2005. Inflammation, atherosclerosis, and coronary artery disease". N. Engl. J. Med. 352 (16), 1685–1695.
Järvisalo, M.J., Harmoinen, A., Hakanen, M., Paakkunainen, U., Viikari, J., Hartiala, J., 2002. Elevated serum C-reactive protein levels and early arterial changes in healthy children". Arterioscler. Thromb. Vasc. Biol. 22 (8), 1323–1328.
Joseph, L., Fink, L.M., Hauer-Jensen, M., 2002. Cytokines in coagulation and thrombosis: a preclinical and clinical review". Blood Coagul. Fibrinolysis 13 (2), 105–116.
Lambalk, C.B., Banga, F.R., Huirne, J.A., Toftager, M., Pinborg, A., Homburg, R., 2017. GnRH antagonist versus long agonist protocols in IVF: a systematic review and meta-analysis accounting for patient type. Hum. Reprod. Update 23 (5), 560–579.
Leppänen, J., Randell, K., Schwab, U., Pihlajamäki, J., Keski-Nisula, L., Laitinen, T., et al., 2020. The effect of different estradiol levels on carotid artery distensibility during a long agonist IVF protocol". Reprod. Biol. Endocrinol. 18 (1), 44–51.
Libby, P., 2012. Inflammation in atherosclerosis. Arterioscler. Thromb. Vasc. Biol. 32 (9), 2045–2051.
Mendelsohn, M.E., 2002. Protective effects of estrogen on the cardiovascular system. Am. J. Cardiol. 89 (12A), 12E–18E.
Moens, A.L., Goovaerts, I., Claeys, M.J., Vrints, C.J., 2005. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? Chest 127 (6), 2254–2263.
Mullen, M.J., Kharbanda, R.K., Cross, J., Donald, A.E., Taylor, M., Vallance, P., 2001. Heterogenous nature of flow-mediated dilatation in human conduit arteries in vivo: relevance to endothelial dysfunction in hypercholesterolemia". Circ. Res. 88 (2), 145–151.
Orvieto, R., Chen, R., Ashkenazi, J., Ben-Haroush, A., Bar, J., Fisch, B., 2004. C-reactive protein levels in patients undergoing controlled ovarian hyperstimulation for IVF cycle. Hum. Reprod. 19 (2), 357–359.
Pacchiarotti, A., Selman, H., Valeri, C., Napoletano, S., Sbracia, M., Antonini, G., 2016. Ovarian stimulation protocol in IVF: an up-to-Date review of the literature". Curr. Pharm. Biotechnol. 17 (4), 303–315.
Pearson, T.A., Mensah, G.A., Alexander, R.W., Anderson, J.L., Cannon, R.O., Criqui, M., et al., 2003. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association". Circulation 107 (3), 499–511.
Persson, M., Ekerfelt, C., Jablonowska, B., Jonsson, Y., Ernerudh, J., Jenmalm, M.C., et al., 2012. Immunological status in patients undergoing in vitro fertilisation: responses to hormone treatment and relationship to outcome". J. Reprod. Immunol. 96 (1–2), 58–67.
Puder, J.J., Blum, C.A., Mueller, B., De Geyter, C., Dye, L., Keller, U., 2006. Menstrual cycle symptoms are associated with changes in low-grade inflammation". Eur. J. Clin. Invest. 36 (1), 58–64.
Raitakari, O.T., Celermajer, D.S., 2000. Testing for endothelial dysfunction". Ann. Med. 32 (5), 293–304.
Saarelainen, H., Valtonen, P., Punnonen, K., Laitinen, T., Raitakari, O.T., Juonala, M., et al., 2009. Flow mediated vasodilation and circulating concentrations of high sensitive C-reactive protein, interleukin-6 and tumor necrosis factor-alpha in normal pregnancy–the Cardiovascular Risk in Young Finns Study". Clin. Physiol. Funct. Imaging 29 (5), 347–352.
Saxena, A.R., Seely, E.W., Goldfine, A.B., 2012. Cardiovascular risk factors and menstrual cycle phase in pre-menopausal women". J. Endocrinol. Invest. 35 (8), 715–719.
Schächinger, V., Britten, M.B., Zeiher, A.M., 2000. Prognostic impact of coronary vasodilator dysfunction on adverse long-term outcome of coronary heart disease". Circulation 101 (16), 1899–1906.
Stoner, L., Erickson, M.L., Young, J.M., Fryer, S., Sabatier, M.J., Faulkner, J., et al., 2012. There's more to flow-mediated dilation than nitric oxide". J. Atheroscler. Thromb. 19 (7), 589–600.
Straub, R.H., 2007. The complex role of estrogens in inflammation". Endocr. Rev. 28 (5), 521–574.
Tostes, R.C., Nigro, D., Fortes, Z.B., Carvalho, M.H., 2003. Effects of estrogen on the vascular system". Braz. J. Med. Biol. Res. 36 (9), 1143–1158.
Williams, M.R., Westerman, R.A., Kingwell, B.A., Paige, J., Blombery, P.A., Sudhir, K., et al., 2001. Variations in endothelial function and arterial compliance during the menstrual cycle". J. Clin. Endocrinol. Metab. 86 (11), 5389–5395.