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Original Article

Application of non-invasive detection of peripheral vascular dysfunction in ovarian hyperstimulation syndrome (OHSS): A pilot study of clinical relevance

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ABSTRACT

Objective: The current study tested the hypothesis that vascular endothelial function, as reflected by the reactive hyperemia index (RHI), and biochemical factors, including VEGF, TNF α , CRP, inhibin A, and inhibin B, were involved in the pathogenesis of ovarian hyperstimulation syndrome (OHSS).**Materials and methods:** This study was conducted between June 2010 and June 2012, enrolling 15 patients with OHSS and 6 healthy control subjects <45 years of age. Detailed clinical parameters were reviewed, including serum VEGF, TNF α , CRP, inhibin A, inhibin B, and hematocrit. RHI assessed by novel automatic peripheral arterial tonography was used to evaluate the vascular endothelial function.**Results:** Twenty-one subjects were evaluated. There was no significant difference between patients with OHSS and control subjects with respect to VEGF, TNF α , CRP, inhibin A and inhibin B. The RHI was not significantly different between patients with OHSS and control subjects (mean, 1.8 ± 0.4 vs. 1.7 ± 0.2). The hematocrit was significantly different between patients with OHSS and control subjects.**Conclusions:** Our preliminary data did not reveal direct evidence of vascular endothelial dysfunction in patients with OHSS. To identify whether RHI could reflect vascular endothelial dysfunction in patients with OHSS, more cases with different severities of OHSS should be recruited in the future study.© 2019 Taiwan Association of Obstetrics & Gynecology. Publishing services by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Introduction

Ovarian hyperstimulation syndrome (OHSS) is an exaggerated response to ovulation induction therapy. Clinicians who prescribe ovulation-inducing agents must be prepared to recognize and manage OHSS [1]. The syndrome of OHSS has a broad spectrum of clinical manifestations, including oliguria, ascites, pleural effusion, hemoconcentration, electrolyte imbalance, liver dysfunction and thromboembolism [2]. The clinical onset of symptoms typically occurs 3–7 days after triggering ovulation with the administration of human chorionic gonadotropin (hCG) or 9–14 days after oocyte retrieval associated with pregnancy. The levels of severity in OHSS vary. Most patients resolve spontaneously within several days;

nevertheless, it can lead to serious morbidity due to thromboembolic events [3,4].

The symptoms are induced by increased vascular permeability, resulting in a fluid shift from the intravascular space to third space compartments [5,6]. Factors that have been implicated in the process include increased secretion or exudation of protein-rich fluid from enlarged ovaries or peritoneal surfaces [7–10], increased follicular fluid levels of prorenin and renin [11,12], and angiotensin-mediated changes in capillary permeability [13]. In addition to increased vascular permeability, arterial vasodilatation is proposed having a role in the underlying pathogenesis of OHSS [14,15]. However, the proposal was mostly based on speculation of hemodynamic parameters. There was only one study with direct clinical evidence, measuring cutaneous vasoconstrictor response via laser Doppler fluximetry [16].

Reactive hyperemia index (RHI) assessed by high-resolution brachial artery ultrasound scanning (BAUS) is a non-invasive, widely applicable peripheral endothelial function test [17]. When exposed to the increased flow and shear stress induced by the

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release of nitric oxide, relaxation of a conduit artery reflects a normal endothelium-dependent process [17,18]. Using this physiologic phenomenon, transient reactive hyperemia will be induced after a brief period of arterial occlusion. Then, RHI can be calculated as one of the markers to evaluate the endothelial function [19,20]. Several studies had reported that lower RHI is a predictor of cardiovascular disease [21,22]. Besides, patients with polycystic ovary syndrome (PCOS), who were also high-risk group of OHSS, were found to have increased RHI [23]. However, this imaging examination for RHI is largely operator-dependent. In current study, we employed a novel automatic and computerized peripheral arterial tonometry (PAT) to obtain the RHI without intra-observer and inter-observer variability. The close correlation between BAUS and PAT was validated previously [24].

To determine the linkage between OHSS and vascular endothelial dysfunction, we examined the non-invasive peripheral arterial tonometry and measured RHI as an indicator of vascular endothelial function. Besides, we also explored the associations among the expression of VEGF, TNF α , CRP, inhibin A, and inhibin B in patients with OHSS.

Materials and methods

Study population

This study was conducted between June 2010 and June 2012, enrolling 15 consecutive patients <45 years of age who were diagnosed with OHSS at the infertility center of Linkou Chang Gung Memorial Hospital. All patients provided informed consent to the study procedures, which were reviewed and approved by the Institutional Review Board of Chang Gung Memorial Hospital. The severity of OHSS was categorized according to the classification proposed by Navot et al. [25]. Inclusion criteria included clinical evidence with hospitalized documentation of OHSS. Informed consent was provided to obtain, analyze, and store blood samples. Exclusion criteria included patients with systemic diseases, such as hypertension, congestive heart failure, diabetes mellitus, cerebral vascular accidents, or coronary artery disease. Age- and gender-matched healthy controls undergoing routine assisted reproductive technology treatment (similar ovulation stimulation protocol with study group) in the same hospital were recruited. None of the controls had systemic disease. The patient records were reviewed for demographic data and medical histories. The two groups of patients were referred to the Vascular Laboratory for vascular ultrasound at the onset of OHSS (same timing in the luteal phase).

Immunoassays for measurement of blood biochemical markers

Peripheral venous blood samples were collected from patients with OHSS and the control group. The samples were measured by enzyme-linked immunosorbent assay (ELISA) using the Human

VEGF ELISA Development System (catalog #DVED00; R&D Systems, Minneapolis, MN, USA). All samples were run in duplicate, and a standard curve was established for each assay. The sensitivity of the assay was <9.0 pg/ml. The intra-assay coefficients of variation were 6.7% at 53.7 pg/ml, 4.5% at 235 pg/ml, and 5.1% at 910 pg/ml ($n = 20$). The inter-assay coefficients of variation were 8.8% at 64.5 pg/ml, 7.0% at 250 pg/ml, and 6.2% at 1003 pg/ml ($n = 40$). Using the Human TNF α ELISA Development System (catalog #HSTA00D; R&D Systems), the minimum detection limit of the assay was 0.5 pg/ml. The intra-assay coefficients of variation were 8.5% at 1.96 pg/ml, 4.3% at 11.5 pg/ml, and 3.1% at 22.1 pg/ml ($n = 20$). The inter-assay coefficients of variation were 10.6% at 1.83 pg/ml, 7.3% at 10.5 pg/ml, and 7.4% at 20.3 pg/ml ($n = 41$). The CRP ELISA kit (catalog #DCRP00; R&D Systems) was used with the detection limitations 0.010 ng/ml. The intra-assay coefficients of variation were 4.4% at 4.79 ng/ml, 3.8% at 8.66 ng/ml, and 8.3% at 18.9 ng/ml ($n = 20$). The inter-assay coefficients of variation were 6.0% at 4.84 ng/ml, 7.0% at 8.66 ng/ml, and 6.6% at 17.5 ng/ml ($n = 40$). The Human Inhibin B ELISA kit (catalog #E01I0005; BlueGene Biotech, Putuo District, Shanghai, China) was used with the sensitivity of 1.0 pg/mL. The inter- and intra-assay variations were <10%. The Human Inhibin A ELISA kit (catalog #E01I0100; BlueGene Biotech, Putuo District, Shanghai, China) was used with the sensitivity of 1.0 pg/mL. The inter- and intra-assay variations were <10%. The manufacturer's recommendations were followed precisely for all immunoassays.

Measurement of vascular endothelial function

All study subjects were referred to the Vascular Laboratory in the morning after fasting overnight for at least 8 h after the onset of OHSS. Tobacco, caffeinated beverages, and any medications, including topical drugs, were prohibited before examination. The PAT signals were obtained by EndoPAT 2000 system (Itamar Medical Inc., Caesarea, Israel) and the whole procedure was described before [26]. Briefly, two pneumatic probes that produce a constant pressure to bilateral distal index fingers were used to continuously measure the beat-to-beat pulsatile volume changes. The applied counter pressure prevented venous pooling thus avoiding venoarterial reflex vasoconstriction without arterial blood flow occlusion.

The reactive hyperemic protocol consists of a 5 min baseline recording, followed by another 5 min occlusion of right brachial blood flow by right arm pressure cuff inflation up to 200 mmHg or 60 mmHg above baseline systolic blood pressure to confirm the PAT signal to zero. After releasing the pressure cuff, the hyperemic PAT tracing was continuously recorded for final 5 min. The RHI was defined as the ratio of average pulse wave amplitude (PAW) during a 1-minute period after cuff release 60 s later compared with baseline average PAW (Fig. 1). In order to decrease confounding variables, this ratio was then normalized to the concurrent signal from left index probe by the system. The signal during the whole

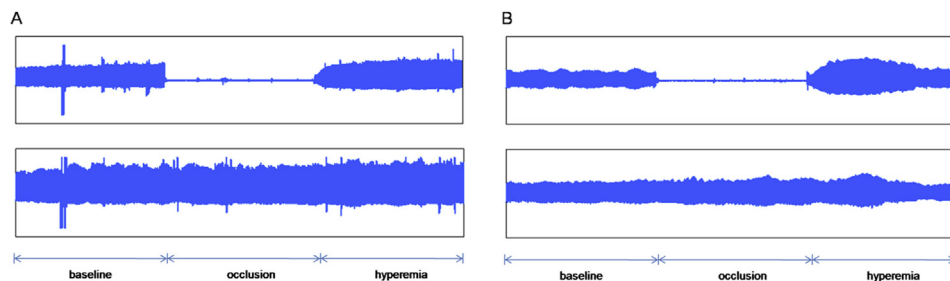


Fig. 1. Example of the PAT tracings. (A) Upper panel shows insignificant reactive hyperemic response of right index finger with reference signal from left index finger at lower panel. The calculated RHI was 1.26. (B) shows a significant hyperemic response after occlusion at upper panel with reference left index finger signal at lower panel. RHI of this case was 1.85.

procedure was automatic recorded and analyzed through a computer algorithm without any inter-observer or intra-observer variability.

Statistical analysis

Differences in the concentrations of hematocrit, VEGF, TNF α , CRP, inhibin A, and inhibin B in serum were determined by Student's *t*-test. In all cases, a *P* value < 0.05 was considered statistically significant. Data are expressed as the mean (range) for continuous variables and as a percentage for categorical variables. Continuous variables were compared among the OHSS and control groups by the non-parametric Mann-Whitney test. The *post hoc* power was also calculated because of the small sample size.

Results

Clinical findings

Fifteen patients with OHSS and 6 healthy control subjects <45 years of age (range, 27–43 years) were analyzed. There were no significant differences between OHSS group and control group with respect to age, body height, weight, and body mass index (Table 1). In the OHSS group, number of retrieved oocytes was 20.1 ± 9.8 (range 6–35), serum hematocrit was 47.0 ± 4.2 (range 39.6–54.1), and peak estradiol level was 1998.3 ± 1644.8 (range 575–5767) (Table 1).

Vascular endothelial function

Brachial artery endothelium-dependent RHI, as an indicator of vascular endothelial function, can be measured non-invasively by high-resolution ultrasound imaging to describe the association between OHSS and endothelial function. The mean RHI was 1.8 ± 0.4 and 1.7 ± 0.2 in the OHSS and control groups, respectively. No significant difference was found (Fig. 2, Table 2).

The biochemical parameters, including VEGF, TNF α , CRP, inhibin A and inhibin B

The serum concentrations of VEGF (mean, 748.9 ± 547.5 vs. 518.7 ± 313.3 pg/ml; Table 2, Fig. 3a), TNF α (mean, 3.9 ± 5.6 vs. 2.0 ± 2.5 pg/ml; Table 2, Fig. 3b), CRP (mean, 5189.4 ± 11055 vs. 6232.7 ± 8995.0 ng/ml; Table 2, Fig. 3c), inhibin A (mean, 130.4 ± 71.6 vs. 98.8 ± 67.7 pg/ml; Table 2, Fig. 4a) and inhibin B (mean, 32.6 ± 22.1 vs. 81.8 ± 152.3 pg/ml; Table 2, Fig. 4b) were not significantly different between patients with OHSS and control subjects.

Discussion

In the field of artificial reproductive technology, OHSS is a common iatrogenic complication. About 5–7% patients would encounter this unpleasant syndrome [27], especially in patients

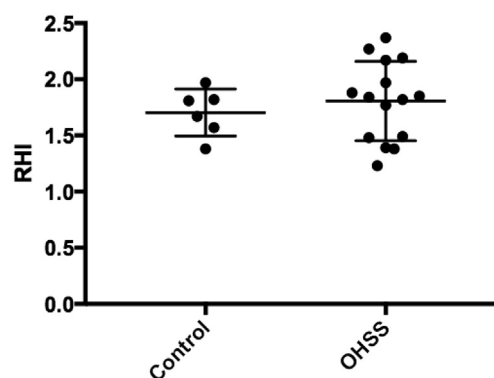


Fig. 2. Vascular endothelial function. The mean RHI was 1.8 ± 0.4 and 1.7 ± 0.2 in the OHSS and control groups, respectively. The RHI was not significantly different between patients with OHSS and control subjects.

with young age or polycystic ovarian syndrome [28,29]. The pathophysiology mostly attributed to increased capillary permeability [6,30], resulting in a fluid shift from the intravascular space to third space compartments. Nevertheless, there are still details to be clarified. Other vascular dysfunction, such as abnormal arterial function, as part of the process had been discussed [14, 16]. However, direct evidence is still lacking. In this study, we conduct a pilot study to investigate the correlation between OHSS and vascular dysfunction by applying peripheral arterial tonometry.

The physiology of the brachial artery RHI is a response to ischemic/hypoxic challenge, followed by anatomic vasodilation, which implies a compensatory increase in the blood flow to repair the transient damage induced by the noxious physiologic challenge. Hyperemia induced by transient ischemia of the forearm increases the shear stress on the brachial artery vessel wall, which provokes endothelial nitric oxide release and subsequently causes vasodilation [18]. We recruited patients with OHSS <45 years of age to avoid age-induced vascular endothelial dysfunction. The mean RHI was 1.8 ± 0.4 and 1.7 ± 0.2 in the OHSS and control groups, respectively. The RHI was not significantly different between patients with OHSS and control subjects (Table 1 and Fig. 2). This result might attribute to small sample size and OHSS severity of the patients enrolled. In the OHSS group, 12 of all 15 patients were classified as moderate-type OHSS, and the other 3 were classified as moderate to severe-type OHSS (Details in Supplementary Table 1). None of them were diagnosed having severe or critical-type OHSS [25]. Therefore, we supposed that the pathophysiology in moderate OHSS was mainly local vascular endothelial dysfunction. The dysfunction might expand to systemic effect along with progression to severe or critical OHSS. To confirm our speculation, further study with larger sample size and patients of different OHSS severities was necessary.

Table 2

Reactive hyperemia index (RHI) and biochemical parameters between OHSS and control group.

Parameters ^a	OHSS (15)	Control (6)	P value
RHI	1.8 ± 0.4	1.7 ± 0.2	NS
VEGF	748.9 ± 547.5	518.7 ± 313.3	NS
TNF- α	3.9 ± 5.6	2.0 ± 2.5	NS
CRP	5189.4 ± 11055	6232.7 ± 8995.0	NS
Inhibin A	130.4 ± 71.6	98.8 ± 67.7	NS
Inhibin B	32.6 ± 22.1	81.8 ± 152.3	NS

^a Mean \pm SD, NS: not significant.

Table 1

Clinical data for OHSS and control group.

Variables ^a	OHSS (15)	Control (6)	P value
Age	31.5 ± 4.01	34.83 ± 5.8	NS
Body height	160 ± 3.7	159.7 ± 3.3	NS
Body weight	60.5 ± 8.9	58.3 ± 11.18	NS
BMI	23.6 ± 3.4	22.8 ± 3.6	NS
Hct	45.1 ± 5.3	39.1 ± 3.5	<0.05

^a Mean \pm SD, NS: not significant.

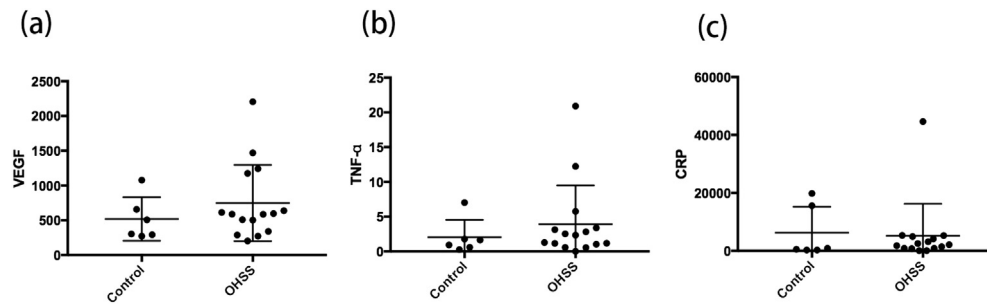


Fig. 3. The biochemical parameters (VEGF, TNF- α , CRP). (a) The serum concentrations of VEGF (mean, 748.9 ± 547.5 vs. 518.7 ± 313.3 pg/ml), (b) TNF- α (mean, 3.9 ± 5.6 vs. 2.0 ± 2.5 pg/ml), (c) CRP (mean, 5189.4 ± 11055 vs. 6232.7 ± 8995.0 ng/ml), were not significantly different between patients with OHSS and control subjects.

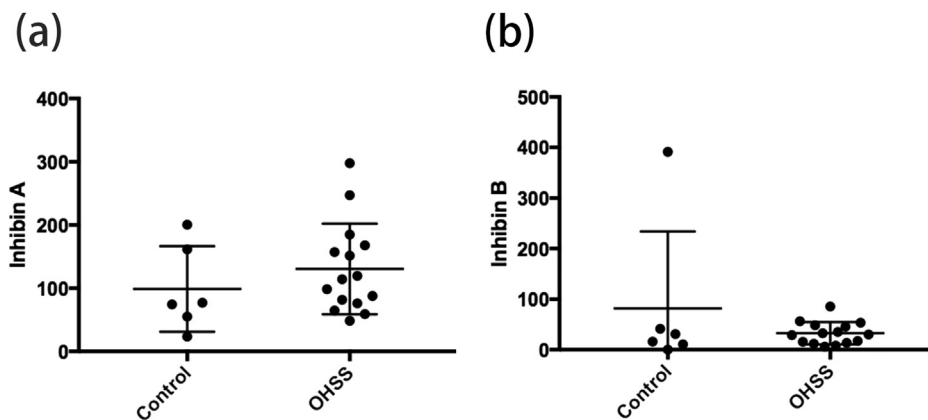


Fig. 4. The biochemical markers (inhibin A, inhibin B) in patients with OHSS and the controls. (a) The serum concentration of inhibin A (mean, 130.4 ± 71.6 vs. 98.8 ± 67.7 pg/ml) was not significantly different between patients with OHSS and control subjects. (b) The serum concentration of inhibin B was not significantly different between patients with OHSS and control subjects (mean, 32.6 ± 22.1 vs. 81.8 ± 152.3 pg/ml).

Currently, supporting management is the mainstay treatment for OHSS in progress. Delineation for signal pathways in endothelial cells would facilitate the use of medications targeting various sites. Multiple biochemical factors, including VEGF, TNF α , CRP, inhibin A, and inhibin B, also known as vascular endothelial function factors, are thought to be involved in the pathophysiology of OHSS. Experiments were conducted in the current study to demonstrate that endothelial function and biochemical factors are involved in the pathogenesis of OHSS. The abundant VEGF in pre-ovulatory human follicular fluid has been shown to increase capillary permeability [6]. Previous reports also demonstrated that serum levels of VEGF in OHSS groups were significantly higher than the controls [31,32]. Although the difference in serum levels of VEGF between the controls and patients who developed OHSS did not reach statistical significance, we still observed higher serum levels of VEGF in the OHSS groups (Table 1 and Fig. 3a). TNF α has a vasodilatory effect on vascular smooth muscle [33]. The vasodilatory effect produced by TNF α is characterized by normal cardiac output, low systemic vascular resistance, and decreased ejection fraction [34]. TNF α released from the ovary may act with granulosa/luteal cell receptors to modulate hormonal effects. This hyperdynamic circulation in OHSS may resemble some of the known effects of TNF α . In the current study, the serum concentration of TNF α was not significantly different between patients with OHSS and control subjects (mean, 3.9 ± 5.6 vs. 1.9 ± 2.3 pg/ml); however, we still observed higher serum levels of TNF α in the OHSS groups (Table 1 and Fig. 3b).

Several ovarian folliculogenesis-related proteins (inhibin A and inhibin B) have been examined to elucidate the process of

folliculogenesis in preliminary experiments [35]. Inhibin secretion by granulosa cells and suppression of FSH production by ovarian inhibin A and inhibin B have been reported [36]. Inhibins are heterodimeric glycoprotein hormones consisting of one α (18 kDa) and one β (14 kDa) chain linked by a disulphide bond. Inhibin A consists of α - β α subunits and inhibin B consists of α - β β subunits [37]. In preliminary experiments, the serum concentration of both inhibin A (mean, 130.4 ± 71.6 vs. 98.8 ± 67.7 pg/ml; Fig. 4a) and inhibin B (mean, 32.6 ± 22.1 vs. 81.8 ± 152.3 pg/ml; Fig. 4b) are not significantly different between patients with OHSS and control subjects. The patients with OHSS tend to have lower level of inhibin A and higher level of inhibin B compared to control group. The differences between inhibin A and inhibin B in patients with OHSS are probably because of the fact that the serum levels display a different time course during the menstrual cycle [38]. While inhibin A increases at the time of the LH surge and stays elevated after oocyte retrieval, inhibin B increases gradually following the FSH stimulation of the granulosa cells and declines at the time of oocyte retrieval. Serum concentrations of inhibin A and inhibin B have no association or only a weak association with OHSS.

As the first pilot study applying peripheral arterial tonometry on patients with OHSS, our preliminary data did not reveal direct evidence of vascular endothelial dysfunction in patients with OHSS. Small sample size and similar OHSS severity of enrolled patients in this study might affect our findings. To identify whether RHI could reflect vascular endothelial dysfunction in patients with OHSS, further study with larger sample size and patients with different severities of OHSS was necessary.

Conflicts of interest

The authors have nothing to disclose.

Supplementary Table 1
Classification in patients with OHSS

Patient	Clinical features	Biochemical features	Classification
1	Symptoms, Acites	Hct 54.1% WBC 21600/uL Albumin 2.68 g/dL	Moderate
2	Symptoms, Acites	Hct 48.1% WBC 20200/uL	Moderate
3	Symptoms, Acites	Hct 50.6% WBC 21600/uL Albumin 3.43 g/dL	Moderate
4	Symptoms, Acites	Hct 40.6% WBC 28900/uL Albumin 3.2	Moderate to Severe
5	Symptoms, Acites	Hct 41.9% Albumin 3.05 g/dL	Moderate
6	Symptoms, Acites	Hct 47.8% WBC 26200/uL	Moderate to Severe
7	Symptoms, Acites	Hct 48.2% WBC 20400/uL Albumin 2.87	Moderate
8	Symptoms, Acites	Hct 50.6% WBC 25100/uL Albumin 3.24 g/dL	Moderate to Severe
9	Symptoms, Acites	Hct 52.9% WBC 22100/uL	Moderate
10	Symptoms, Acites	Hct 39.6%	Moderate
11	Symptoms, Acites	Hct 45.4% WBC 22600/uL Albumin 3.19 g/dL	Moderate
12	Symptoms, Acites	Hct 44.7% WBC 16900/uL	Moderate
13	Symptoms, Acites	Hct 48.1% WBC 23600/uL	Moderate
14	Symptoms, Acites	Hct 46.8% Albumin 3.47 g/dL	Moderate
15	Symptoms, Acites	Hct 46%	Moderate

References

- [1] Blankstein J, Shalev J, Saadon T, Kukia EE, Rabinovici J, Pariente C, et al. Ovarian hyperstimulation syndrome: prediction by number and size of pre-ovulatory ovarian follicles. *Fertil Steril* 1987;47(4):597–602.
- [2] Delvigne A, Rozenberg S. Review of clinical course and treatment of ovarian hyperstimulation syndrome (OHSS). *Hum Reprod Update* 2003;9(1):77–96.
- [3] Sachar P, Rajamani K. Young ischemic stroke in association with ovarian hyperstimulation syndrome. *J Stroke Cerebrovasc Dis* : Off J Natl Stroke Assoc 2016;25(9):e134–40.
- [4] Mor YS, Schenker JG. Ovarian hyperstimulation syndrome and thrombotic events. *Am J Reprod Immunol (New York, NY : 1989)* 2014;72(6):541–8.
- [5] Tollan A, Holst N, Forsdahl F, Fadnes HO, Oian P, Maltau JM. Transcapillary fluid dynamics during ovarian stimulation for in vitro fertilization. *Am J Obstet Gynecol* 1990;162(2):554–8.
- [6] Goldsman MP, Pedram A, Dominguez CE, Ciuffardi I, Levin E, Asch RH. Increased capillary permeability induced by human follicular fluid: a hypothesis for an ovarian origin of the hyperstimulation syndrome. *Fertil Steril* 1995;63(2):268–72.
- [7] Bergh PA, Navot D. Ovarian hyperstimulation syndrome: a review of pathophysiology. *J Assist Reprod Genet* 1992;9(5):429–38.
- [8] Koninckx PR, Heyns W, Verhoeven G, Van Baelen H, Lissens WD, De Moor P, et al. Biochemical characterization of peritoneal fluid in women during the menstrual cycle. *J Clin Endocrinol Metab* 1980;51(6):1239–44.
- [9] Koninckx PR, Renaar M, Brosens IA. Origin of peritoneal fluid in women: an ovarian exudation product. *Br J Obstet Gynaecol* 1980;87(3):177–83.
- [10] Donnez J, Langerock S, Thomas K. Peritoneal fluid volume and 17 beta-estradiol and progesterone concentrations in ovulatory, anovulatory, and postmenopausal women. *Obstet Gynecol* 1982;59(6):687–92.
- [11] Sealey JE, Atlas SA, Glorioso N, Manapat H, Laragh JH. Cyclical secretion of prorenin during the menstrual cycle: synchronization with luteinizing hormone and progesterone. *Proc Natl Acad Sci U S A* 1985;82(24):8705–9.
- [12] Derckx FH, Alberda AT, Zeilmaker GH, Schalekamp MA. High concentrations of immunoreactive renin, prorenin and enzymatically-active renin in human ovarian follicular fluid. *Br J Obstet Gynaecol* 1987;94(1):4–9.
- [13] Lightman A, Tarlatzis BC, Rzasz PJ, Culler MD, Caride VJ, Negro-Vilar AF, et al. The ovarian renin-angiotensin system: renin-like activity and angiotensin II/III immunoreactivity in gonadotropin-stimulated and unstimulated human follicular fluid. *Am J Obstet Gynecol* 1987;156(4):808–16.
- [14] Balasch J, Fabregues F, Arroyo V. Peripheral arterial vasodilation hypothesis: a new insight into the pathogenesis of ovarian hyperstimulation syndrome. *Hum Reprod (Oxford, England)* 1998;13(10):2718–30.
- [15] Balasch J, Arroyo V, Carmona F, Llach J, Jimenez W, Pare JC, et al. Severe ovarian hyperstimulation syndrome: role of peripheral vasodilation. *Fertil Steril* 1991;56(6):1077–83.
- [16] Foong LC, Bhagavath B, Kumar J, Ng SC. Ovarian hyperstimulation syndrome is associated with reversible impairment of vascular reactivity. *Fertil Steril* 2002;78(6):1159–63.
- [17] Moens AL, Goovaerts I, Claeys MJ, Vrints CJ. Flow-mediated vasodilation: a diagnostic instrument, or an experimental tool? *Chest* 2005;127(6):2254–63.
- [18] Corretti MC, Anderson TJ, Benjamin EJ, Celermajer D, Charbonneau F, Creager MA, et al. 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* 2002;39(2):257–65.
- [19] Anderson EA, Mark AL. Flow-mediated and reflex changes in large peripheral artery tone in humans. *Circulation* 1989;79(1):93–100.
- [20] Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID, et al. Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. *Lancet* 1992;340(8828):1111–5.
- [21] Paine NJ, Hinderliter AL, Blumenthal JA, Adams Jr KF, Sueta CA, Chang PP, et al. Reactive hyperemia is associated with adverse clinical outcomes in heart failure. *Am Heart J* 2016;178:108–14.
- [22] Schoenenberger AW, Urbanek N, Bergner M, Toggweiler S, Resink TJ, Erne P. Associations of reactive hyperemia index and intravascular ultrasound-assessed coronary plaque morphology in patients with coronary artery disease. *Am J Cardiol* 2012;109(12):1711–6.
- [23] Raja-Khan N, Shuja SA, Kunselman AR, Hogeman CS, Demers LM, Gnatuk CL, et al. Brachial artery conductance during reactive hyperemia is increased in women with polycystic ovary syndrome. *Eur J Obstet Gynecol Reprod Biol* 2011;155(1):49–53.
- [24] Kuvin JT, Patel AR, Sliney KA, Pandian NG, Sheffy J, Schnall RP, et al. Assessment of peripheral vascular endothelial function with finger arterial pulse wave amplitude. *Am Heart J* 2003;146(1):168–74.
- [25] Navot D, Bergh PA, Laufer N. Ovarian hyperstimulation syndrome in novel reproductive technologies: prevention and treatment. *Fertil Steril* 1992;58(2):249–61.
- [26] Axtell AL, Gomari FA, Cooke JP. Assessing endothelial vasodilator function with the Endo-PAT 2000. *JoVE* 2010;(44).
- [27] Tarlatzis BC, Griesinger G, Leader A, Rombauts L, Ijzerman-Boon PC, Mannaerts BM. Comparative incidence of ovarian hyperstimulation syndrome following ovarian stimulation with corifollitropin alfa or recombinant FSH. *Reprod Biomed Online* 2012;24(4):410–9.
- [28] Luke B, Brown MB, Morbeck DE, Hudson SB, Coddington 3rd CC, Stern JE. Factors associated with ovarian hyperstimulation syndrome (OHSS) and its effect on assisted reproductive technology (ART) treatment and outcome. *Fertil Steril* 2010;94(4):1399–404.
- [29] Delvigne A, Demoulin A, Smitz J, Donnez J, Koninckx P, Dhont M, et al. The ovarian hyperstimulation syndrome in in-vitro fertilization: a Belgian multicentric study. I. Clinical and biological features. *Hum Reprod (Oxford, England)* 1993;8(9):1353–60.
- [30] Rodewald M, Herr D, Duncan WC, Fraser HM, Hack G, Konrad R, et al. Molecular mechanisms of ovarian hyperstimulation syndrome: paracrine reduction of endothelial claudin 5 by hCG in vitro is associated with increased endothelial permeability. *Hum Reprod (Oxford, England)* 2009;24(5):1191–9.
- [31] Agrawal R, Tan SL, Wild S, Sladkevicius P, Engmann L, Payne N, et al. Serum vascular endothelial growth factor concentrations in in vitro fertilization cycles predict the risk of ovarian hyperstimulation syndrome. *Fertil Steril* 1999;71(2):287–93.
- [32] Wang TH, Horng SG, Chang CL, Wu HM, Tsai YJ, Wang HS, et al. Human chorionic gonadotropin-induced ovarian hyperstimulation syndrome is associated with up-regulation of vascular endothelial growth factor. *J Clin Endocrinol Metab* 2002;87(7):3300–8.
- [33] Hollenberg SM, Cunnion RE, Parrillo JE. The effect of tumor necrosis factor on vascular smooth muscle. In vitro studies using rat aortic rings. *Chest* 1991;100(4):1133–7.
- [34] Natanson C, Eichenholz PW, Danner RL, Eichacker PQ, Hoffman WD, Kuo GC, et al. Endotoxin and tumor necrosis factor challenges in dogs simulate the cardiovascular profile of human septic shock. *J Exp Med* 1989;169(3):823–32.
- [35] Woodruff TK, Mather JP. Inhibin, activin and the female reproductive axis. *Annu Rev Physiol* 1995;57:219–44.
- [36] Erickson GF, Hsueh AJ. Secretion of “inhibin” by rat granulosa cells in vitro. *Endocrinology* 1978;103(5):1960–3.
- [37] Vale W, Rivier C, Hsueh A, Campen C, Meunier H, Bicsak T, et al. Chemical and biological characterization of the inhibin family of protein hormones. *Recent Prog Horm Res* 1988;44:1–34.
- [38] Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP, et al. Measurement of dimeric inhibin B throughout the human menstrual cycle. *J Clin Endocrinol Metab* 1996;81(4):1401–5.