Association between Follicle Stimulating Hormone (FSH), Vascular Endothelial Growth Factor (VEGF), and Caspase-3 of Follicular Fluid with The Number of Mature Oocytes of Patients underwent In Vitro Fertilization Program

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Citation

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Abstract

Background: Determine the levels of FSH, VEGF, and Caspase-3 in follicular fluid and the correlation with the number of mature oocytes of IVF program patients.

Methods: Measurement of FSH, VEGF, and Caspase-3 in follicular fluid of patients who underwent IVF was done.

Results: We found higher FSH level [(6.44 vs 2.16 ng/mL (p<0.015) and lower VEGF level [(2229.28 ± 841.8 vs 3566.77 ± 432.50 pg/ml (p<0.001)] of follicular fluid in the group of mature oocytes ³5. No significant difference of Caspase-3 level [0.09 vs 0.08 ng/ml (p=0.430)] was found in mature oocytes <5 and ³5 group, respectively. We found significant positive correlation of FSH levels (r 0.657, p<0.001), significant negative correlation of VEGF levels (r -0.656, p<0.001), and no correlation of caspase-3 (r -0.116, p 0.496) with the number of mature oocytes.

Conclusions: Follicular fluid FSH levels were positively correlated, VEGF levels were negatively correlated, and Caspase-3 levels were not correlated with the number of mature oocytes.

INTRODUCTION

Infertility is defined as a failure to establish pregnancy after one year regular sexual intercourse (2-3 times a week) of couple without contraception. In general, the prevalence of infertile woman aged 15-49 years old in Indonesia is approximately 4.5-6%. Some of those infertile couples require in vitro fertilization (IVF) in an assisted reproductive technology (ART) clinic.[1]

Whilst the IVF technology is progressively developed, pregnancy and livebirth rates are low. The success of IVF is determined by all process from the beginning of the preparation phase, controlled ovarian stimulation, oocyte retrieval, fertilization, the number of embryo produced, to the process of embryo transfer into the uterus. Thus, the number of mature oocytes retrieved and its quality is essential for the ability of oocytes to be fertilized, for the embryo quality, and successful implantation when oocytes are being transferred into the uterus.[1]

Currently, evaluation of mature oocytes for IVF is done by microscopic morphological evaluation, which is very subjective. An additional objective method is required as an adjunct to evaluate oocyte quality. Biochemical evaluation of follicular fluid has been studied to indicate how many mature oocytes can be obtained from an IVF program.[1]

Follicular fluid contains any amount of follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotrophin (hCG), transforming growth factor (TGF)-b, and other growth factors such as vascular endothelial growth factor (VEGF), interleukin, reactive oxygen species (ROS), apoptotic factor, protein, amino acid, glucose, and prostanoid. Biochemical analysis of follicular fluid is able to indirectly provide information on the oocyte quality, its potential to be fertilized, and good embryo development in either normal reproductive cycle or IVF program.2,3

VEGF is an essential substance in the follicular fluid for neovascularization around the follicle to transfer hormones and maintain oxygen availability for granulosa cell and oocytes.[3] Whether VEGF positively or negatively correlates with the number and oocyte quality, fertilization rate, and the number of embryo produced is still debatable. Some studies stated that high level of VEGF increase granulosa cell apoptosis and affect follicle development, low oocyte maturity, fertilization rate and embryo implantation. Another studies demonstrate evidence of high VEGF level as a marker of high quality oocytes.[4,5]

The ability of granulosa cell to proliferate is achieved through an equilibrium state between growth and apoptosis. Apoptotic rate negatively correlates with the quality of oocytes, and further affects fertilization rate and the number of embryo.[5-7]

Apoptosis occurs through extrinsic or intrinsic pathway such as hypoxia. The intrinsic pathway can also be induced by a lack of growth factors, hormones, and cytokines that inhibit cell death. As consequence, caspase-9 and further caspase-3 eventually will be activated to induce activation of the caspase pathway for apoptosis. Follicular fluid with elevated level of caspase-3 is associated with greater number of granulosa cell undergoes apoptosis, and thus will affect oocyte maturity.[8-10]

The aim of this study was to determine the relationship between the levels of FSH, VEGF, and Caspase-3 in follicular fluid with the number of mature oocytes, fertilization rate and percentage of embryo of patients underwent IVF program.

MATERIALS AND METHODS

This was an analytical cross-sectional study among 37 patients undergoing an IVF program with a long GnRH agonist protocol at the IVF clinics of the Harapan Kita Women and Children Hospital from June 2013 to March 2014.

We included patients: women age £42 years old, with complete basic infertility evaluation, with clear indication for IVF, measured basal FSH level <15 IU/L, and the number of antral follicle being ³8. Participants not willing to participate and only join partial IVF program were excluded from the study.

MEASUREMENT OF FSH, VEGF, AND CASPASE-3 LEVELS IN FOLLICULAR FLUID

FSH level was assessed with enzyme-amplified chemiluminescence (Immulite). Measurement of VEGF level was carried out with enzyme-linked immunosorbent assay reagent kit (R&D System, Minneapolis, Minnesota, USA) with detection threshold 9.0 pg/mL. While Caspase-3 level measurement was conducted with ELISA (Cell Signalling Tech. Human cleaved Caspase-3 at Asp 175 ELISA kit, Beverly, MA, USA).

STATISTICAL ANALYSIS

Shapiro Wilk test for normally distributed numeric data, unpaired t-test and Mann-Whitney for bivariate analysis, and Pearson correlation were used for the statistical analysis. Multivariate analysis was used to control confounding factors. All of the data analysis was conducted with SPSS ver. 13.0 for windows. P£ 0.05 was considered as significant, with the power of 95%.

RESULTS

Table 1 summarizes the characteristic of 37 patients. The groups were defined according to the number of mature oocytes. Significant differences of the mean age between patients with mature oocytes ³5 and patients with mature oocytes <5 was found with p<0.001, as well as the basal FSH level differences with p=0.019.

Table 1

Difference of age and basal FSH level

Variable	Mature Oocytes		P-value
	≥ 5 (n=28)	< 5 (n=9)	
Age (years)			**<0.001[a]**
Mean(SD)	33.82±3,40	39.67±1.73	
Basal FSH			**0.019[b]**
Median	6.45	10.30	
Range	(4.10-12.30)	(5.40-13.20)	

[a] Unpaired t-test; [b] Mann-Whitney test

The FSH and VEGF levels of follicular fluid had significant differences with the number of mature oocytes (Table 2). Higher FSH levels were found in follicular fluid among patients with mature oocytes ³5. In contrast, lower VEGF levels were found in this group. The Caspase-3 level of the follicular fluid did not differ significantly between these groups.

Table 2

Difference of FSH, VEGF, and Caspase-3 level of follicular fluid

Variable	Mature Oocytes		P-value
	≥ 5 (n=28)	< 5 (n=9)	
FSH (ng/ml)			**0.015[b]**
Median	6.44	2.16	
Range	1.26-21.68	1.38-10.41	
VEGF (pg/ml)			**<0.001[a]**
Mean(SD)	2229.28(841.81)	3566.77(432.50)	
Caspase-3 (ng/ml)			**0.430[b]**
Median	0.08	0.09	
Range	0.05-4.19	0.07-4.06	

[a] Unpaired t-test; [b] Mann-Whitney test.

Follicular fluid levels of FSH had a significant positive correlation with the number of mature oocytes (p<0.001) (Table 3). A negative significant correlation between the VEGF levels in follicular fluid and number of mature oocytes was seen (p<0.001). Meanwhile, the Caspase-3 level in follicular fluid did not correlate significantly with the number of mature oocytes (p 0.496).

Table 3

Correlation of follicular fluid FSH, VEGF, and Caspase-3 levels with the number of mature oocytes

	Correlation coefficient	P-value
FSH (ng/ml)	0.657	<0.001
VEGF (pg/ml)	-0.656	<0.001
Caspase-3 (ng/ml)	-0.116	0.496

Bivariate analysis, Pearson correlation test

DISCUSSION

In this study, the mean age of patients undergoing an IVF program in the IVF clinics at the Harapan Kita Women and Children Hospital with the mature oocytes ≥5 was younger than the other group. This finding was consistent with the physiological decline of reproductive function as the women are getting older. Reduced oocyte storage constantly occurs in women during reproductive age, but the process occurs more progressive by the age of 35 until 40 years old, in particular, during the time of being 37-38 years old. The remaining oocytes were approximately 25000 and less than 1000 follicles at menopause.[11] The patients' age is an important determinant factor of stimulation response or mature oocytes obtained in IVF program.

Basal FSH level is commonly used as an indicator of oocytes storage and to predict ovarian response. The oocytes storage is reduced when the basal FSH level is elevated. As the oocytes storage is reduced, a low ovarian response to stimulation will occur, as shown in this study. In contrast, Koo et al. found no differences between basal FSH level with low or normal stimulation response.[12] The different finding possibly was due to the genetic factor or different research methods.

We found significant higher FSH levels of follicular fluid in the mature oocytes ³5 group compared to the other group. High FSH levels of follicular fluid has been reported to increase the number of mature oocytes obtained in an IVF program. This is due to the oocytes growth that occurred mainly in the pre-antral follicle. These follicles are selected under the effect of FSH and subsequently increase in size and specialized granulosa cell become cumulus and mural granulosa cells. It demonstrates the effect of FSH on the number of selected follicles and eventually will affect the number of mature oocytes produced.[13]

A significant positive correlation between the FSH level of follicular fluid and the number of mature oocytes was revealed in this study. The higher the FSH level, the more number of mature oocytes can be obtained in an IVF program.

The FSH of follicular fluid will give positive impact on granulosa cell proliferation and oocyte maturity, will increase the number of embryos and the successful rate of pregnancy in an IVF program. The level is affected by the dose of gonadotrophin given and hypophysis suppression. FSH hormone also contributes to the process of cellular death and granulosa cell apoptosis. This hormone plays synergistic role with estradiol to increase the ovum cytoplasmic maturity through cyclic AMP secretion and controls oocytes during meiosis.[14]

An elevated VEGF level of follicular fluid is an indicator of low oocytes quality. This study showed the negative correlation between VEGF level of follicular fluid and the number of mature oocytes. This was in line with a study that concluded negative correlation in IVF patients with natural cycle, clomiphene citrate stimulation, as well as hMG gonadotrophin stimulation.[15] Similar findings were also reported in a study with a combined GnRH agonist protocol among the low and normal response groups. Low ovarian response to controlled ovarian hyper-stimulation and an increased number of granulosa cell apoptosis with high VEGF levels in the follicular fluid were reported.[5] In contrast, a study from Benifla et al. concluded that the

VEGF level of the follicular fluid could not be suggested as an indicator to predict the successfulness of an IVF program, in particular, among patients under the age of 40 years.[16]

Direct correlation of follicular fluid VEGF level with the blood flow was stated in a study by Monteleone et al., which showed an increase in fertilization rate, good quality of embryos, and higher pregnancy rate.[17] Negative correlation was also found between VEGF levels of the follicular fluid and the number of oocytes, peak concentration of serum estradiol and pregnancy rate, although there was a positive correlation between follicular fluid VEGF levels and older age.[18,19] Hypoxic condition was also a significant factor in increased VEGF levels of the follicular fluid.[18] However, no significant difference between the VEGF level of follicular fluid and patient's age was reported in another study.[16]

Vascular endothelial growth factor (VEGF) is produced by granulosa and theca cells as the response to FSH, LH, hCG, and hypoxia. In physiological conditions, VEGF induces angiogenesis around a follicle to ensure the availability of hormonal, nutrient, and oxygen for follicle growth and development. In hypoxic circumstances, VEGF will be produced excessively as a compensatory mechanism. In spite of the difficulty in determining of how much VEGF concentration is needed in physiologic and pathological conditions, the VEGF level tends to increase in follicular fluid when hypoxia exists as well as among patients with older reproductive age.[20-23]

We found no differences as well as no significant correlation between the level of follicular fluid caspase-3 and the number of mature oocytes. It was comparable with the report from Suh et al. who found no significant differences between the expression of granulosa cell apoptosis and the number of mature oocytes, although they said the degree of apoptosis could be used as prognostic indicator to assess the outcome of an IVF program.[24] Oxidative stress will induce granulosa cell apoptosis as proven in animal study. The GnRH agonist also can affect granulosa cell apoptosis.[25]

With the presence of cell death signal, the pro-apoptotic protein modifies post-translation that will activate and translocate mitochondria to induce apoptosis. As the response to an apoptotic stimulus, the outer membrane of mitochondria will become permeable and induce cytochrome-C release, a caspase inducer. Once the caspase is activated, the process of cell death cannot be avoided. Cytochrome-C will enter the cytosol, interact with Apaf-1, and induce the activation of caspase-9 proenzyme. Caspase-9 activation then activates caspase-3 as the executor and further induces activation the remaining caspase cascade and as a result, apoptosis occurs. Caspase activation will break nuclear lamine and decompose the nucleus by caspase-3.[8,9,26-28]

CONCLUSION

In our study, we found that follicular fluid FSH levels were positively correlated and VEGF levels were negatively correlated with the number of mature oocytes. We were also unable to find a correlation of follicular fluid caspase-3 with the number of mature oocytes in our IVF program. All of those substance levels were also not associated with the number of mature oocytes obtained in the IVF program. The results indicated that there is a difference in response of stimulation that likely is affected by an individual genetic factor. Therefore, further research is needed to study the adjustment of a controlled ovarian stimulation protocol based on individual genetic and physiological characteristic. We also suggest that follicular fluid VEGF level are used as an additional indicator to predict the number of mature oocytes obtained from oocyte retrieval in order to improve the successfulness of an IVF program.

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