Correlation Among Follicle-stimulating Hormone, Insulin-like Growth Factor-I and Aromatase Expression with Oocyte Maturation in an Assisted Reproductive Program

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Citation


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Abstract

Background: Follicular fluid plays important roles in oocyte quality, which can increase the success rate of assisted reproductive technology (ART) program. Oocyte maturity is influenced by the follicular fluid’s chemical component, including insulin-like growth factor (IGF)-I, which increases follicle-stimulating hormone (FSH) receptor; thereby increasing levels of intracellular cyclic adenosine monophosphate (cAMP) as primary mediator of FSH stimulation in aromatase expression.

Materials and Methods: This was an analytical correlational study with a cross-sectional approach in ART patients program at Dr. Hasan Sadikin Hospital Bandung. The p <0.05 considered as significance.

Results: Mature and immature oocytes showed significant differences in FSH expression (p=0.03). The relationship of IGF-I and oocyte maturity showed no statistical significance (p=0.192). The median ratio of mature oocytes to total oocytes was 0.71; those with a median prevalence ratio > 0.71 showed strong FSH expression, which 1.71-fold higher than those with a lower proportion of mature oocytes. The aromatase expression in the follicular fluid of those patients with a median prevalence ratio of mature oocytes > 0.71 was 1.55-fold higher than in those with lower proportion of mature oocytes, who exhibited aromatase-negative-expression in the follicular fluid.

Conclusion: Higher aromatase expression results in higher IGF-I and FSH expression in follicular fluid.

INTRODUCTION

Infertility rates continue to increase worldwide; based on demographic and health survey by the World Health Organization in 2012, the burden of infertility is still high in women. In the period from 1990-2010, the number of couples with infertility increased from 42 million to 48.5 million [1]. In a demographic and health survey from the period 1994-2000, the percentage of Indonesian women (aged 15-49 years) with infertility was approximately 10.2% [2]. The success of assisted reproductive technology (ART) as reflected by the rate of implantation, pregnancy and childbirth is still approximately 30% [3,4]. Follicle growth monitoring and reproductive hormonal level evaluation are integral parts of the ART program. The number and quality of oocytes reflects the success of the program. Therefore, the assessment of ovarian function to predict the number and quality of oocytes is very important [5].

Chemical elements of follicular fluid have been grouped into several categories, including a) hormones (follicle-stimulating hormone or FSH, leutinizing hormone or LH, human chorionic gonadotropin or hCG), b) growth factors of the transforming growth factor-beta (TGF-beta) superfamily, c) other growth factors (insulin-like growth hormone), and d) reactive oxygen species (ROS) [6]. Several animal studies have studied the expression of aromatase and chemical elements in follicular fluid as markers of oocyte quality, including FSH and insulin-like growth factor (IGF)-I [6,7], but until now, research on human follicular fluid has not
been performed. FSH is a very important hormone in the mammalian reproductive system. This hormone is necessary for gonadal development and maturation at puberty, and for gamete production during the fertile phase. The degree of FSH influence on follicular development is still not well established [8]. Furthermore, the expression of FSH influences the expression of the FSH receptor that involves IGF-I.

IGF is a small peptide resembling pro-insulin with an estimated molecular mass of 7.5 kDa and has an important role in the process of folliculogenesis. IGF-I also plays a role in granulosa and theca cell stimulation, DNA synthesis, steroidogenesis, aromatase activity, LH receptor synthesis, and inhibin secretion. IGF-I works in synergy with FSH to stimulate protein synthesis and steroidogenesis. In the follicle, IGF-I is believed to originate from blood circulation rather than from local production. IGF-I in human ovarian follicles is the primary IGF, acting as an FSH action mediator that stimulates steroidogenesis in granulosa cells [9,10].

FSH does not regulate the expression of IGF-I but significantly increases FSH receptor (FSHR) expression, thus creating feedback loops involving IGF-I, FSH and FSHR. Furthermore, FSH induces an increase in intracellular cyclic adenosine monophosphate (cAMP) levels, which is the main mediating mechanism by which FSH stimulation affects aromatase expression. The expression of aromatase reaches its peak in the pre-ovulatory follicle. In this phase, we found that oocytes showing second polar bodies had matured [11]. This study was conducted by analyzing the components of the follicular fluid (FSH, IGF-I and aromatase expression) of humans associated with oocyte maturity, that helps to improve the success rate of ART.

MATERIALS AND METHODS

The subjects of this study were all female patients who followed the ART program at the Aster Fertilization Clinic at Dr. Hasan Sadikin Hospital (RSHS) in Bandung in the period from January 2011 to February 2012. This study was a correlational analytical study with a cross-sectional approach in patients who followed the ART program at RSHS. Immunohistochemistry (IHC) analysis was done using antibodies targeting FSH, IGF-I and aromatase. Staining was performed using a streptavidin-biotin method as described previously [12]. In brief, paraffin blocks were processed into 4-μm-thick sections and were then incubated with fresh 0.3% hydrogen peroxide in methanol for 30 min at room temperature. After rinsing in phosphate-buffered saline (PBS; pH 7.4), nonspecific binding sites were blocked by incubating with 10% normal serum for 30 min. The specimens were then incubated with anti-FSH (ab80839), anti-IGF-I (ab9572) or anti-aromatase (ab18995) Abcam polyclonal antibodies. The staining was scored semi-quantitatively as follows: total staining was scored as the staining intensity (on a scale of 1 + to 3+) and distribution of staining; level 3+ was the strongest and 1+ was the lowest staining intensity. A distribution of < 20% was scored 1, 20-50% was scored 2, 50-80% was scored 3 and > 80% was scored 4. Statistical analysis was performed using SPSS, ver. 16.0. P-values < 0.05 were significant.

RESULTS

Patient characteristics and expression of FSH, IGF-I, and aromatase in follicular fluid

A medical history was collected from all subjects. Patients’ characteristics including age and duration of infertility, and a clinical examination to determine the causes of infertility was performed. In addition, all subjects underwent IHC examination for FSH, IGF-I and aromatase expression in the follicular fluid. The mean age of patients in this study was 33.2 years, with a range of 26-41 years, and 41% of subjects (21 people) were between 30-34 years of age. Infertility was present for 6-10 years in 47% of subjects (23 people). Among the causes of infertility in this study population, the sperm factor was the most common (43%, 21 people), followed by the tubal factor (27%, 13 people). Table 1 shows the IHC examination of the follicular fluid. The percentages of subjects that expressed FSH, IGF-I and aromatase in the follicular fluid were 10% (6 persons), 16% (8 persons) and 12% (6 persons), respectively.

Examination of Mature and Immature Oocytes Based on FSH Expression in ART

Table 1 shows the numbers of mature and immature oocytes based on FSH expression. Analysis of variance (ANOVA) examining the difference in the average number of oocytes between those expressing and those not expressing FSH in the follicular fluid revealed a p value = 0.495 for mature oocytes and a p = 0.089 for immature oocytes. The Wilcoxon test revealed that the average number of mature oocytes differed significantly based on FSH expression (p = 0.003), with high FSH expression associated with a higher mean mature oocyte count vs immature oocyte.
Correlation Among Follicle-stimulating Hormone, Insulin-like Growth Factor-I and Aromatase Expression with Oocyte Maturation in an Assisted Reproductive Program

Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative (n=20)</th>
<th>Weak (n=9)</th>
<th>Moderate (n=5)</th>
<th>Strong (n=6)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I</td>
<td>3.9 (2.9)</td>
<td>3.8 (2.5)</td>
<td>5.2 (1.6)</td>
<td>5.3 (1.9)</td>
<td>0.405</td>
</tr>
<tr>
<td>Median</td>
<td>3</td>
<td>3</td>
<td>6</td>
<td>5.5</td>
<td>-</td>
</tr>
<tr>
<td>Range</td>
<td>0-11</td>
<td>0-8</td>
<td>3-7</td>
<td>2-7</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative (n=20)</th>
<th>Weak (n=9)</th>
<th>Moderate (n=5)</th>
<th>Strong (n=6)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH</td>
<td>2.34 (2.9)</td>
<td>2.32 (3.3)</td>
<td>5.20 (1.1)</td>
<td>1.17 (1.2)</td>
<td>0.689</td>
</tr>
<tr>
<td>Median</td>
<td>1.00</td>
<td>2.00</td>
<td>5.0</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>Range</td>
<td>0-10</td>
<td>0-10</td>
<td>4-7</td>
<td>0-3</td>
<td>-</td>
</tr>
</tbody>
</table>

Examination of Mature and Immature Oocytes Based on IGF-I Expression in ART

Table 2 shows the numbers of mature and immature oocytes based on IGF-I IHC expression. Analysis of variance (ANOVA) examining the difference in the average number of oocytes between those expressing and those not expressing IGF-I in the follicular fluid was p = 0.488 for mature oocytes and p = 0.464 for immature oocytes, revealing no significant differences. The Wilcoxon test revealed a significant difference (p < 0.05) in the mean number of mature relatives to immature oocytes in those with negative and those with moderate IGF-I expression (p < 0.05). The results showed that those with moderate IGF-I expression expressed a higher average number of mature oocytes than immature oocytes.

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Negative (n=20)</th>
<th>Weak (n=9)</th>
<th>Moderate (n=5)</th>
<th>Strong (n=6)</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatase</td>
<td>2.7 (2.7)</td>
<td>5.0 (2.4)</td>
<td>5.9 (2.7)</td>
<td>4.5 (2.1)</td>
<td>0.550</td>
</tr>
<tr>
<td>Median</td>
<td>4</td>
<td>6</td>
<td>4.5</td>
<td>4.5</td>
<td>-</td>
</tr>
<tr>
<td>Range</td>
<td>0-11</td>
<td>1-7</td>
<td>2-10</td>
<td>2-7</td>
<td>-</td>
</tr>
</tbody>
</table>

Examination of Mature and Immature Oocytes Based on Aromatase Expression in ART

Table 3 shows the numbers of mature and immature oocytes based on aromatase IHC expression. There were no significant differences (p > 0.05) in the mean number of mature or immature oocytes across those with different amounts of aromatase expression. The Wilcoxon test revealed that the average number of mature oocytes in those with moderate levels of aromatase expression in the follicular fluid was significantly higher (p = 0.032) than the average number of immature oocytes.

Correlation of IGF-I, FSH, and aromatase expression with patient age

Table 5 show that the expression of IGF-I, FSH and aromatase wasn’t significantly correlated with the age of the study subjects (p > 0.05); further analysis of the study age subject wasn’t performed.

Correlation of IGF-I, FSH, and aromatase expression with infertility factors

As shown in Table 6, the expression levels of IGF-I, FSH, and aromatase were not significantly associated with the causes of infertility (p > 0.05); therefore, further analysis of the cause of infertility was not performed.

Correlation of IGF-I, FSH and aromatase expression in the follicular fluid with number of oocytes

Table 7 shows that the number of mature oocytes was positively correlated with FSH and aromatase expression with rs = 0.222 and rs = 0.206, respectively. These results show that an increase in FSH and aromatase expression increases the number of mature oocytes. High IGF-I expression resulted in fewer mature oocytes, although this effect was not significant.
Number of Mature and Immature Oocytes in Research Subjects

Table 8 showed the mean numbers of mature and immature oocytes were 4.2 and 2.6 with a median of 4 and 1, respectively. The number of mature oocytes ranged from 0-11, and the number of immature oocytes ranged from 0-10. The mean proportion of mature oocytes to total oocytes was 0.63, with a median of 0.71 and a range from 0-1.0. To measure the correlation between IGF-I, FSH, and aromatase expression in the follicular fluid with oocyte maturity, we measured the prevalence ratio with a 95% confidence interval. The median value of mature oocytes relative to total oocytes was 0.71.

Correlation of IGF-I, FSH and Aromatase Expression in Follicular Fluid with Oocyte Maturity

Table 9 shows that oocyte maturity was significantly associated with the expression of FSH. Those with strong FSH expression exhibited a 1.71-fold greater chance of having mature oocytes than those with weak or no FSH expression.

Correlation of IGF-I, FSH and Aromatase Expression in the Follicular Fluid Based on Oocyte Maturity

Table 10 shows the variables studied in those patients with a mature oocyte ratio > 0.71 and in those with a mature oocyte ratio <0.71. The three variables observed (IGF-I, FSH and aromatase expression) were significantly different between these two groups (p <0.05). The correlation of IGF-I expression with FSH and aromatase expression in those patients with a mature oocyte ratio > 0.71 was stronger than the correlation in those with a mature oocyte ratio <0.71.
Correlation Among Follicle-stimulating Hormone, Insulin-like Growth Factor-I and Aromatase Expression with Oocyte Maturation in an Assisted Reproductive Program

Supplement 3

Supplement table 1

Supplement table 2

Supplement table 3

Supplement table 4
Correlation Among Follicle-stimulating Hormone, Insulin-like Growth Factor-I and Aromatase Expression with Oocyte Maturation in an Assisted Reproductive Program

Supplement table 5

Table 5: Number of Mature and Immature Oocytes in Research Subjects

<table>
<thead>
<tr>
<th>Oocyte Number</th>
<th>Statistic Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>1. Mature</td>
<td>4</td>
</tr>
<tr>
<td>2. Immature</td>
<td>2</td>
</tr>
<tr>
<td>3. Immature</td>
<td>6</td>
</tr>
</tbody>
</table>

Supplement table 6

Table 6: Correlation of IGF-I, FSH and Aromatase Expression in Follicular Fluid with Oocyte Maturity

<table>
<thead>
<tr>
<th>Variables</th>
<th>Maturity Median Prevalence ( \geq 0.71 ) (n=25)</th>
<th>( \leq 0.71 ) (n=22)</th>
<th>p-value</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IGF-I expressions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>5</td>
<td>3</td>
<td>0.08</td>
<td>0.35(0.15-1.74)</td>
</tr>
<tr>
<td>Moderate</td>
<td>3</td>
<td>2</td>
<td>0.04</td>
<td>0.48(0.16-1.39)</td>
</tr>
<tr>
<td>Weak</td>
<td>4</td>
<td>1</td>
<td>0.06</td>
<td>0.46(0.15-1.40)</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>12</td>
<td>0.005</td>
<td>1.03(0.23-5.43)</td>
</tr>
<tr>
<td>FSH expressions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>6</td>
<td>2</td>
<td>0.01</td>
<td>0.71(0.28-2.20)</td>
</tr>
<tr>
<td>Moderate</td>
<td>5</td>
<td>1</td>
<td>0.01</td>
<td>0.56(0.26-1.22)</td>
</tr>
<tr>
<td>Weak</td>
<td>3</td>
<td>6</td>
<td>0.02</td>
<td>0.69(0.21-2.28)</td>
</tr>
<tr>
<td>Negative</td>
<td>17</td>
<td>12</td>
<td>0.10</td>
<td>1.03(0.21-5.43)</td>
</tr>
<tr>
<td>Aromatase expressions</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strong</td>
<td>2</td>
<td>4</td>
<td>0.01</td>
<td>0.69(0.21-2.28)</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>2</td>
<td>0.01</td>
<td>0.69(0.21-2.28)</td>
</tr>
<tr>
<td>Weak</td>
<td>4</td>
<td>2</td>
<td>0.01</td>
<td>0.69(0.21-2.28)</td>
</tr>
<tr>
<td>Negative</td>
<td>14</td>
<td>15</td>
<td>0.01</td>
<td>1.03(0.21-5.43)</td>
</tr>
</tbody>
</table>

DISCUSSION

In this study, the average age of patients who followed the ART program at Aster Fertility Clinic RSHS was 33.2 years, with a range of 26-41 years, with 41% (21 individuals) in the range of 30-34 years). Most subjects were aware of the infertility for 6-10 years (47%, 23 people). This long duration of infertility may be due to delays in fertile couples seeking treatment for infertility and the current tendency of women to delay marriage. For the cause of infertility in this study, the most common factor was sperm factor (43%, 21 patients). Female patients having tubal factor (27%, 13), with 16% (8 people) of the sample having endometriosis. These rates are similar to those reported in the literature, which has shown that male factor was the most common form of infertility [13,14].

In this study, some samples showed negative expression for aromatase, IGF-I and FSH. These samples were than considered as negative control, and given no primary antibody. In this study, the comparison of the mean number of mature oocytes and immature oocytes based on FSH expression showed that there was a significant difference (\( p = 0.003 \)); those with strong FSH expression exhibited more mature oocytes than immature oocytes. The comparison of the mean number of mature and immature oocytes based on IGF-I expression showed that there was a significant difference in those patients with negative and moderate IGF-I expression (\( p < 0.05 \)). The results showed that the average number of mature oocytes was higher than the mean number of immature oocytes in these patients. The difference in the mean number of mature and immature oocytes did not appear to differ based on aromatase expression (\( p > 0.05 \)). However, there were more mature oocytes than immature oocytes in those with moderate aromatase expression.

In this study, there was a significant correlation between oocyte maturity with FSH expression. Those with strong FSH expression exhibited a 1.71-fold higher chance of having mature oocytes than those with weak or no FSH expression (Table 4.10). The results of Silva JM and CA Price [15] on cow granulosa in vitro concluded that an increase in FSH would increase aromatase expression. Other results were found in Guyansyah A. et al. [16], which suggested that there was a significant relationship between FSH and oocyte quality (\( p = 0.03 \)). These observations further indicate that FSH is the main inducer of aromatase activity in granulosa cells. In vitro studies have shown that androgens increase FSH-stimulated steroidogenesis stimulated by increasing cAMP levels [17]. In mice, androgen receptor expression is at its highest in the early antral and preantral phases, then decreases gradually as follicles mature, while aromatase expression simultaneously increases. These findings suggest that androgens increase FSH in the early stages of follicular development, but during the late stages of follicular development, they function as a substrate for estrogen synthesis [17,18]. Further follicular development depends on the action of FSH on FSHR.
expressed by granulosa cells.

A study by Zhou J, et al. [19], showed a highly significant positive correlation between IGF-I expression in granulosa cells and DNA synthesis. Because IGF-I increases the proliferation of different cell types, and IGF-I receptors are expressed with IGF-I in ovarian follicles, IGF-I appears to act in autocrine / paracrine fashions to stimulate granulosa cells. The mechanism by which IGF-I is selectively induced in a group of dominant follicles remains unknown. With these considerations, FSH is suspected to stimulate follicle growth by inducing the production of IGF-I in granulosa cells [15]. This result explains the relationship between FSH action and IGF-I expression in rat ovaries when the expression of FSHR and IGF-I in serial ovarian sections is compared during their cycle, lactation, and pregnancy [19]. In all ovaries, FSHR mRNA was detected only in IGF-I mRNA-positive follicles, whereas IGF-I receptor mRNA was found in all follicles. To determine whether FSH induces IGF-I expression in granulosa cells, IGF-I expression was evaluated in FSH knockout mice. Ovarian FSH knockout mice exhibited a similar selective distribution of IGF-I mRNA and the same number of granulosa cells as that in wild-type mice.

Furthermore, Zhou J et al. evaluated IGF-I, FSHR and aromatase expression levels after hypophysectomy in the presence or absence of gonadotropin (PMSG) replacement. Aromatase mRNA is used as a functional marker of the effects of FSH. Hypophysectomy in the presence or absence of gonadotropin therapy had no effect on IGF-I mRNA-positive follicles, whereas IGF-I receptor mRNA was found in all follicles. To determine whether FSH induces IGF-I expression in granulosa cells, IGF-I expression was evaluated in FSH knockout mice. Ovarian FSH knockout mice exhibited a similar selective distribution of IGF-I mRNA and the same number of granulosa cells as that in wild-type mice.

In subjects undergoing ART, high aromatase expression was correlated with high FSH and IGF-I expression, especially in study of couples that can reproduce voluntarily without restriction in North America. This study showed that fertility decreases with age. The overall infertility rate was 2.4%; 11% of women did not give birth to children after the age of 34 years, 33% at age 40 years, and 87% at age 45 years. Overall, research data in the USA and other populations show that the peak fertility of women is at 20 to 24 years of age, with a relatively small decline up to approximately 30-32 years of age, followed by a progressive decline after 40 years of age. Overall, fertility rates are 4% to 8% lower in women aged 25-29, 15% to 19% lower between the ages of 30 and 34, 26% to 46% lower in women aged 35-39, and as much as 95% lower between the ages of 40 and 45. At menopause, the number of follicles remaining is less than 1,000. The changes that occur before menopause are characterized by elevated FSH levels and decreased estradiol, but LH levels remain stable [21]. With decreasing estradiol levels, the presence of IGF-I in follicular fluid is further reduced. This reduction is thought to be one reason why IGF-I expression was not found in all samples. A study by Tesarik J et al. showed that patients > 40 years old who were given 8 units of growth hormone subcutaneously on day 7 of ovarian stimulation obtained greater sustained pregnancy rates and decreased abortion compared to women aged > 40 years old given a placebo [22].

Limitation of the Study

The results of the above research indicate the limitations encountered during the implementation of the study. These results were less than perfect. The analysis performed in this study was limited to the analysis of the components of the follicular fluid and their association with oocyte maturity. In addition, existing data on variations in the cause of infertility and longevity suggests that an analysis of confounding variables of the cause of infertility and duration would be preferable. However, these limitations could be overcome by multivariable analysis, but the sample size needs to be larger than that examined in our study.

CONCLUSION

FSH expression in the follicular fluid was higher in mature oocytes than in immature ones, similar to aromatase expression, while IGF-I follicular fluid expression was not higher in immature oocytes than in mature oocytes. In subjects undergoing ART, high aromatase expression was correlated with high FSH and IGF-I expression, especially in...
the follicular fluid of mature oocytes. Those with a mature oocyte ratio > 0.71 had strong FSH expression, which was 1.71-fold higher than the FSH expression in those with a mature oocyte ratio <0.71. The expression of aromatase in the follicular fluid of those patients with a median prevalence ratio of mature oocytes > 0.71 was 1.55-fold higher than in those with a lower proportion of mature oocytes, who exhibited negative expression of aromatase in the follicular fluid.

DECLARATIONS

Ethics Approval and Consent to Participate

This study protocol was approved by Faculty of Medicine Ethics Committee Review Board, Universitas Padjadjaran and all study participants gave informed consent, patients consent to participate was written. We hereby declare that all patients have been examined in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Consent to Publish

We declare that written informed consent was obtained from each patient details for publication of this study.

Availability of data and materials section

Authors declare that the personal data of the patients will not be shared, because of patient’s confidentiality.

Competing Interests

We declared that no competing interests exist.

Funding

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Authors’ contributions

WP, TD, AG, AB, JCM and BSH had examined, treated, observed and followed up the subject of this study. LS, AF, KIM correcting and writing the manuscript. All authors has read and approved of the final manuscript.

References

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