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To cite this article: B Wiweko et al 2018 J. Phys.: Conf. Ser. 1073 032052

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**BMP15 mRNA profile in granulosa cells from endometriosis patients undergoing *in vitro* fertilization**

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**Abstract.** Endometriosis is a female reproductive disorder that can cause infertility by lowering oocyte quality due to a low quality of oocytes. Granulosa cell assessment is a minimally invasive procedure for determining oocyte quality. Bone morphogenetic protein 15 (BMP15) also acts as a growth factor for oocyte derivatives that regulate proliferation and granulosa cell differentiation. In endometriosis, higher BMP15 expression is assumed to induce oocyte growth. This study aimed to determine the mRNA levels of BMP15 in granulosa cells from endometriosis patients undergoing *in vitro* fertilization. We analyzed samples from 12 patients with endometriosis and seven normal women using real-time PCR at Yasmin Clinic, Kencana, Cipto Mangunkusumo Hospital. Granulosa cells collected during oocyte retrieval were preserved using RNAlater® QIAGEN and were evaluated by absolute quantitative real-time PCR with pre-manufactured oligonucleotides as the standard. Results showed that although BMP15 did not significantly differ between the endometriosis patients and the controls (p > 0.05), the endometriosis patients had higher BMP15 mRNA levels than the controls. Therefore, further study is warranted to gain more comprehend data.

**1. Introduction**

Endometriosis is a female reproductive disorder that can cause infertility due to a low quality of oocytes [1]. Endometriosis is defined as the presence of glandular and stromal tissue (endometrium-like tissue) in female reproductive organs besides the uterus [2], and it is often diagnosed after difficulty conceiving children. It is assumed that the lower oocyte quality in endometriosis patients is because of the higher apoptosis rates in the granulosa cells surrounding the oocytes [1,3]. These higher apoptosis rates can decrease the levels of nutrients and growth factors needed for oocyte growth, resulting in abnormal oocyte maturation and, thus, lower oocyte quality [4]. Granulosa cell assessment is a minimally invasive procedure for determining oocyte quality. The connection between oocytes and granulosa cells is assumed to indicate oocyte quality.
Bone morphogenetic protein 15 (BMP15) is a member of the TGFβ family, which influences physiology and follicular growth. BMP15 is not only expressed in oocytes, but also in granulosa cells and in follicular liquid [5]. BMP15 protein also acts as a growth factor for oocyte derivate, which regulates the proliferation and differentiation of granulosa cells. In humans, the BMP15 concentration in woman which has intracytoplasmic sperm injection is associated with fertility level [6,7]. The higher the BMP15 concentration in follicular, the higher the fertility level [8]. Women with a heterozygote mutation in the BMP15 gene are diagnosed as infertile because of ovarian hypergonadotropic failure [9]. BMP15 mutation is also related to iatrogenic ovarian hyperstimulation syndrome induced by over FSH response [8,10]. In endometriosis cases, elevated BMP15 expression can induce oocyte growth. Therefore, we aimed to compare BMP15 expression between the endometriosis patients and the normal controls to improve the diagnosis and treatment of endometriosis diagnosis.

2. Methods

2.1. Samples

The samples were granulosa cells collected from 12 endometriosis patients undergoing in vitro fertilization (IVF) and seven normal women (control). All the samples were collected from IVF Laboratorium, Yasmin Kencana Clinic, Cipto Mangunkusumo Hospital. The granulosa cells were stored in tubes and were preserved using RNAlater® QIAGEN (Hilden, Germany).

The Health Research Ethics Committee, Faculty of Medicine Universitas Indonesia-Cipto Mangunkusumo Hospital approved this study, and informed consent was obtained from all the participants.

2.2. RNA isolation and cDNA synthesis

The RNA was isolated using QIAamp RNA Blood Mini Kit (QIAGEN) based on the QIAamp Blood Mini Handbook protocol, with modifications. A Spectrophotometer NanoDrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA) was used to measure the RNA purity and concentration. cDNA was synthesized from RNA samples using a QIAGEN QuantiTect Reverse Transcription Kit based on the protocol in the QuantiTect Reverse Transcription Mini Handbook, with some modifications. The program to synthesize the cDNA was as follows: 42 °C for 30 min and 95 °C for 10 min. Then, the samples were stored at −20 °C until analysis.

2.3. Real-time PCR measurements

Gene Q Real-Time PCR (QIAGEN) was used to measure BMP15 expression. We used the following primer pairs for BMP15: 5’ GGAGTTCATGGAAGGGAATCT 3’ (forward) and 5’ for 15 min; 94 °C for 15 s; 60 °C for 30 s, and 72 °C for 30 s. We used a standard curve to quantify BMP15 expression in all the samples. The standard curve was determined using oligonucleotide G-Blocks to substitute the cDNA sample. G-blocks with known concentrations were diluted to $10^{-1}$, $10^{-2}$, $10^{-4}$, $10^{-6}$, and $10^{-8}$ ng/µL, and were amplified using real-time PCR; then, the cycle threshold (Ct) value was used to quantify the gene expression in the samples.

2.4. Data analysis

The data were analyzed using SPSS.21 software to compare the mean of R (fold change value) between the chronic phase and the late phase groups. The Kolmogorov–Smirnov test was used to test the normality of the data. To test for significant differences between the two groups, unpaired t-tests were used for the normally distributed data and the Mann–Whitney test was used for the data that was not normally distributed. The significance level was set at p value <0.05.

3. Results

Our results showed a higher BMP15 expression in the endometriosis patients than in the normal women, although this difference was not statistically significant. The BMP15 expression data in this
research was not normally distributed. The maximum and minimum values were 40.16 and 0.10, respectively (data not shown). Although the wide range in these values suggests high individual variation in our samples (Figure 1), the standard deviation showed low individual variation (0.66 in the endometriosis patients and 1.05 in the normal women; Table 1).

![Figure 1. BMP15 expression in study samples](image)

**Figure 1.** BMP15 expression in study samples

| Target Gene | Group                          | R = $2^{-\Delta \Delta C_{P}}$ | p-value |
|-------------|--------------------------------|---------------------------------|---------|
| BMP15       | Endometriosis patients (n = 12)| 0.84 ± 0.66                     | 0.27    |
|             | Normal women (n = 7)           | 0.25 ± 1.05                     |         |

### 4. Discussion

Patients with endometriosis have poor IVF outcomes, including low pregnancy rates, high miscarriage rates, and low live birth rates. These poor outcomes are correlated with oocyte quality, which is defined as the ability of oocytes to complete maturation or undergo successful fertilization. A quality marker of oocytes is BMP15, a member of the TGFβ family, which affects physiology and follicular growth. The BMP15 protein is also a functional growth factor of in-functional oocytes regulation of proliferation and differentiation of granulosa cells. Furthermore, it affects the development of the granulosa cell layer closest to the oocytes, the cumulus cell layer.

Our results are not supported by those of a previous study that reported a positive association between *BMP15* expression and fertility levels. BMP15 is assumed to regulate proliferation and differentiation in granulosa cells [5–7]. BMP15 was also assumed to induce oocyte growth [5]. From
these findings, we can assume higher expression in normal women than in endometriosis patients. It was assumed to indicate higher oocyte quality to develop diagnosis and treatment of endometriosis.

Previous research has shown higher BMP15 mRNA expression in normal human oocytes than in PCOS patients, which conflicts with our findings in the present study showing higher expression in granulosa cells from endometriosis patients than from normal controls. The reason behind this finding is that in PCOS patients, the follicles are arrested at the primordial stage, during which BMP15 is not expressed. However, in endometriosis patients, the LH receptor is disturbed, resulting in delayed maturation of the corpus luteum, thus, increasing prolactin, prostaglandin, and macrophage levels.

In endometriosis patients, BMP15 prevents granulosa cell luteinization and ovulation by inhibiting FSHR expression. Unbalanced exposure of BMP15 may alter follicular sensitivity to FSH, and, thus, could impair the process of dominant follicle selection. Furthermore, treatment of human granulosa cells with BMP family may induce AMH expression at the mRNA and protein levels and increase FSHR expression.

In our study, we found no significant difference in BMP15 mRNA expression between the controls and endometriosis patients. However, we found a correlation between BMP15 in endometriosis patients and the regulation of proliferation, differentiation, apoptosis, luteinization, and the metabolism of adjacent granulosa cells. The lack of significant difference found in this study may have been because the granulosa samples were collected from stimulated ovaries, which may exhibit altered expression levels of oocyte-secreted factors such as BMP15.

Another possibility is that different point mutations in BMP15 affected the results. Patients with some types of BMP15 point mutations can develop lower protein expression. In this study, we did not analyze all the BMP15 point mutations in every sample, resulting in sample bias. Different stages of endometriosis such as moderate to severe endometriosis may also show different BMP15 expression.

The sample size used in this study was small, which may have influenced the results, and more samples are needed to confirm our results. Our small number of samples may also have influenced the data distribution, which would, subsequently, also affect the statistical analysis. Furthermore, many factors can influence BMP15 expression, such as AMH, inhibin, TGFβ, and activin receptor. Additionally, different patient statuses could also have influenced our results.

5. Conclusion
It is concluded that BMP15 expression does not significantly differ between endometriosis patients and normal women. Further study is warranted to gain more comprehend data, and research into other genes related to BMP15 and endometriosis is also needed to confirm the role of BMP15 in endometriosis.

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