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THE RELATIONSHIP BETWEEN GDF-9 AND BMP-15 SERUM AND FOLLICULAR FLUID AND THE QUALITY OF OOCYTES IN WOMEN WHO UNDERGO AN IVF CYCLE

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ABSTRACT

Objective: The quality of the embryo is greatly influenced by the quality of the oocytes; oocyte-secreted factors (OSFs), which include GDF-9 and BMP-15, play an important role in folliculogenesis. This study was to determine the relationship between GDF-9 and BMP-15 serum within the follicular fluid in order to predict the quality of oocytes in women undergoing *In vitro* Fertilization (IVF).

Methods: We collected 30 samples of blood serum and 30 samples of follicular fluid on the day of ovum pickup (OPU), and examined GDF-9 and BMP-15 using ELISA kits. Analysis by Pearson and a partial-correlation was conducted to analyze the correlation between the concentration of GDF-9 and BMP-15 in serum and follicular fluid with general physiological parameters, such as maturation and fertilization rates.

Results: The mean age of the subjects was 35,0(26,0-39,0) years. There was no statistical correlation between GDF-9 serum and follicular fluid (p=0.245); but there was a correlation between BMP-15 serum and follicular fluid (p=0.001). Average level of GDF-9 in the follicular fluid was 163,0 pg/ml (48,0-537), and average level in the serum was 260.33 pg/ml±121,82; average levels of BMP-15 in the follicular fluid was 58.30pg/ml±31,54 and average levels of BMP-15 in the serum was 74.20 pg/ml (1,0–610).

Conclusion: There were no correlations between levels of GDF-9 serum-FF and BMP-15-FF, and maturation rates and fertilization rates. There was a negative correlation between BMP-15 serum and maturation rates.

Keywords: BMP-15, Fertilization rate, Follicular fluid, GDF-9, Maturation rate

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INTRODUCTION

IVF is a procedure wherein fertilization of the egg cell by the sperm is accomplished outside the body *in vitro* as an alternative method in infertility cases where other methods were unsuccessful [1]. Reasons for IVF include abnormalities of the fallopian tubes, unexplained infertility (idiopathic), male infertility, endometriosis and infertility due to cervical factors or immunological and hormonal disorders [2, 3]. According to the Society for Assisted Reproductive Technology, data show that 11% of couples of reproductive age experience infertility, and approximately 10% of such cases require IVF with an average gestation of 21.3% and a birth-rate of 33.1% [4, 5]. Based on this report, Indonesian women who underwent IVF in 2018 achieved pregnancy rates of 29.05%. Since the birth of the first IVF baby on July 25, 1978, IVF has become the most often-used assisted reproductive technology (ART) program, but the IVF process is not easy for couples and yields low success rates [1-5].

The quality of the embryo is greatly influenced by the quality of the oocytes; oocytes that are of poor quality can be the cause of infertility in women and a major obstacle to success with the IVF program. Several factors have been used as parameters to assess oocyte quality; the morphology parameters have much in used to search for a marker of the oocyte quality that can provide better potential for the development of the embryo.

A disadvantage of this system is the multiplicity of methods used in different studies, which sometimes leads to controversial results. Some research indicates a significant relationship between the morphology of the oocyte and the quality of the embryo, while other studies do not show a meaningful relationship. This is due to the nature of an assessment that is subjective and depends on which method embryologists use [5-12].

Assessments of oocyte maturation still use a combination of methods for monitoring the nucleus and the cytoplasm. However, this raises a question as to the presence of other factors that play a role in the rate of oocyte maturation; factors to consider are Anti-Müllerian hormones (AMH), GDF-9 and BMP-15. Several of the endocrine markers for reproductive function are used to manage infertility and estimate potential fertility. Follicle-stimulating hormones (FSH), oestradiol (E2) and AMH are the markers in ovaries that are monitored to assess the likely magnitude of the ovarian response to gonadotropin stimulation during ART, as they provide an indication of the amount of follicular growth in the ovaries. Some of these markers only offer an estimate of ovarian reserve, but no information of 'oocyte quality', which has an impact on limitations related to the fertility of women. BMP-15 and GDF-9 are markers that indicate the function and quality of the oocytes; both are mostly secreted by the oocyte and play an important role in the development of oocytes and the fertility of mammals. BMP-15 and GDF-9 are considered to be factors that are secreted paracrine by oocytes and act in the follicles of the ovaries to regulate the signaling and communication of cells around the oocyte (somatic cells) (i.e., granulosa and cumulus cells that regulate folliculogenesis and fecundity). BMP-15 is an important factor for reproduction in mono-ovular species such as humans and sheep, but not in a poly-ovular species like a rat. GDF-9 is very important for the fertility of mammals in both mono-and poly-ovular species, and mutations that lead to inactivation of the GDF-9 genes result in infertility in sheep and rats [6-14].

GDF-9 and BMP-15 are members of the superfamily of transforming growth factors (TGF) and are secreted from the oocyte during the folliculogenesis process. Both are very important for folliculogenesis and female fertility. Diminishing GDF-9 will cause a decrease in the proliferation of granulosa cells, resulting in abnormal oocyte growth and failure of the follicles to develop beyond the primary stage; GDF-9 also inhibited apoptosis of granulosa cells and atresia of ovarian follicles. BMP-15 stimulates the mitosis and proliferation of granulosa cells; mutation of BMP-15 in women leads to ovarian

hypergonadotropic. Both will inhibit the secretion of progesterone in the end of the follicular phase. Additionally, the oocytes regulate the proliferation of cumulus cells, apoptosis, metabolism and expansion by secreting these two factors paracrine [4–6, 13–32].

Granulosa cells that are directly exposed to the oocyte during the development of follicle and ovulation appear to regulate oocyte development and fertilization and are governed by the oocyte factors. GDF-9 can inhibit premature secretion of progesterone, the formation of receptor LH and the synthesis of cyclic adenosine monophosphate. GDF-9 is the main medium that is secreted by the oocyte, which can stimulate expansion of cumulus cells. In a previous study in patients with polycystic ovary syndrome, even though ovulation stimulation sparks the growth of follicles, the expression of GDF-9 in granulosa cells remains lower than the normal value, which can cause premature luteinization and decrease oocyte development; this may be related to the high rate of miscarriage [8, 9, 12, 33–38].

Assessment of GDF-9 and BMP-15 can be done through the expression of mRNA in granulosa cells, concentrations in the serum and follicular fluid. Although assessment of concentrations of GDF-9 and BMP-15 in the serum is still limited, but from study by Riepsamen *et al.*; the concentration levels of GDF-9 and BMP-15 can already be detected in the serum of women who undergo an IVF course in Australia with the use of monoclonal antibodies as the epitope of the GDF-9 and BMP-15 proteins so that the markers can also be checked through an examination of their respective levels in serum such as AMH [6]. As to the presence of BMP-15 and GDF-9 in serum, they should be exempt from the proteoglycan-binding granulosa/cumulus extracellular matrix (ECM) transit through the basal lamina of the follicle and migrates puzzle to the ECM to enter into the blood vessels. The TGFß superfamily plays an important role in controlling the growth-factor mechanism that transits through the ECM, although it is unclear how this is related to BMP-15, GDF-9 and AMH. Therefore, the magnitude of the BMP-15 and GDF-9 serum may depend on the regulation of the ECM, gap junctions and capillary leakage, so the levels are similar in the ovaries. The presence of specific proteins in the serum represents a potential target in the examination of female fertility. If the levels of AMH serum can determine the reserve of oocytes in the ovaries, then GDF-9 and BMP-15 serum can be a marker of the quality of the oocyte. In the course of examining the concentration of GDF-9 and BMP-15 follicular fluid, there have been studies that show the relationship between the concentration of GDF-9 and BMP-15 in follicular fluid with the oocyte quality [6–8, 39–49].

Assessment of the quality of the oocytes will be seen from the maturation of oocytes (either the nucleus, cytoplasm and the morphology of the polar bodies) and the rate of fertilization has been known as a predictor of fertilization of oocytes and quality of embryos after intracytoplasmic sperm injection (ICSI). In their research, xia viewed the three factors—namely the polar bodies, space perivitelline and body inclusions in the cytoplasm—to assess the quality of oocytes based on the morphological criteria outlined by Veeck (1990) [50–64].

Furthermore, in IVF, the rate of fertilization is commonly used to measure how many oocytes were fertilized by a sperm; normal fertilization is identified by the presence of two pronuclei (2pn) at the time of the fertilization assessment (i.e., 16 h to 19 h after ICSI or conventional insemination). The rate of fertilization is a predictor, which is also a good estimate of the success of the implantation.

The aim of this study is to determine the relationship between the levels of GDF-9 and BMP-15 serum with the levels of follicular fluid to predict the quality of oocytes (i.e., oocyte maturation rates and fertilization rates) in women who are undergoing IVF.

MATERIALS AND METHODS

This study was approved by the Committee of Health Ethics of our university in September 2019. All subjects signed informed consent. A descriptive-analytic study using the designed cross-sectional study was performed at the Centre of Reproductive Medicine in the hospital of our university (Yasmin Clinic). In total, 30 women who underwent the IVF procedure with short protocol for ovarian stimulation and ICSI participated in this study.

Women IVF candidates were 20–40 y of age, were willing to engage in this study and were undergoing an IVF course. Exclusion criteria included patients with polycystic ovarian syndrome and/or endometriosis who had not attained oocyte maturation, and for whom the IVF procedure had been postponed. Demography data of the patients is presented in table 1.

All subjects underwent the protocol for ovarian stimulation. Blood samples were taken to determine basal levels of endocrine hormones during the menstrual cycle. Endocrine hormones such as FSH, LH and E2 were measured using an Axsym Chemiluminescence Detection System (Axsym; Abbott Laboratories, Rungis, France). The regimen of stimulation is Gonadotropin (Gonal-f, 150–300 IU; Merck Serono, Darmstadt, Germany) that was subcutaneously injected for ovarian induction on days 2–3. The regimen of hCG, 10,000 IU (Ovidrel; Merck Serono) was intramuscularly injected when the diameter of a minimum of three dominant follicles was at least 18 mm. Blood samples of 5 cc of blood were collected before ovum retrieval and entered into a serum separator tube, then brought to the integrated laboratory in our faculty. Where the serum samples were standing for two hours until they solidified at room temperature, centrifuged 1000 rpm at a temperature of 2–8 °C, and then stored for 3–4 w at a temperature 4 °C; the supernatant was saved for analysis of GDF-9 and BMP-15. After the ovum retrieval process, embryologists took the intrafollicular fluid and sent it to the laboratory, where the GDF-9 and BM-15 levels were examined using ELISA kits.

Examination of the serum and the follicular fluid was performed with the sandwich ELISA principle technique for the ELISA kits, in which the plate is precoated with specific antibodies for human GDF-9 (mab 72 b) and human BMP-15 (mab 28 a) with the specifications of the reagent/kit (GDF-9 and BMP-15), sensitivity of 0.1 ng/ml and detection range of 0.16–10 ng/ml. The specificity of this kit has been recognized for a sample of GDF-9; there are no cross-reaction or disorder in human or the other substances.

The principle works thus: the sample (serum/follicular fluid) or the standard is added into the micro-ELISA plate and combined with specific antibodies. It is then biotinylated with antibodies to human GDF-9 and BMP-15, and conjugation of avidin-horseradish peroxidase (HRP) is added onto each microplate then incubated. Components free components the other will be leached, and the substrate liquid is added into each of the tubes/wells. Only wells that contained GDF-9/BMP-15 that their antibodies were biotinylated and avidin-HRP which will emit a blue color. The reaction of the enzyme substrate is terminated by adding a stop solution that will turn into a yellow color. Optical density spectrophotometer used for the measurement with a wavelength of 450 nm 2 nm where the optical density is proportional to GDF-9/BMP-15. The concentration of human GDF-9 and BMP-15 can be calculated by comparing the optical density of the sample against the standard. Oocytes from OPU are inspected and analyzed by embryologists at the laboratory of our hospital's IVF clinic to assess the degree of morphological oocytes.

The maturation of oocytes is examined under an inverted microscope. If a first polar body is observed in the oocyte cytoplasm, the oocyte is considered to be in the metaphase II (MII) stage; the oocyte maturation rate refers to the number of MII oocytes divided by the total number of all oocytes at OPU. Regarding the rate of fertilization, normal fertilization is identified by the presence of two pronuclei (2pn) at the time of the fertilization assessment (i.e., 16 h to 19 h after ICSI); the fertilization rates refer to the number of fertilization oocytes divided by the total number of all oocytes at OPU.

Statistical analysis

The normal distribution of all data was examined by the Kolmogorov-Smirnov test, and the data in the tables are shown as means±standard deviation (SD). The correlation between the level of GDF-9 and BMP-15 serum and follicular fluid with several physiological parameters (age, BMI, etc.) was analyzed with the Pearson and Spearman correlation coefficient. The correlation between GDF-9 and BMP-15 levels and oocyte developmental

(maturation rates and fertilization rates) was used for partial analysis. After adjusting for the number of retrieved oocytes, the comparison of GDF-9 and BMP-15 levels in different groups were used for a covariance analysis by linier regression. Data analysis was conducted with the Statistical Program for Social Sciences (SPSS) version 20.0, and statistical significance was considered at P<0.05.

RESULTS

Our result showed that the mean age of the subjects was 35,0(26,0-39,0) years and the mean duration of infertility of the subjects was 6.6±5,31 y. The characteristics/descriptives of the subjects are given in table 1.

Table 1: The characteristics/descriptives of the subjects

Characteristic	n=30
Age (year)	35,0 (26,0-39,0)
Duration of infertlity (year)	6,6 ± 5,31
BMI	24,00(18,8-35,6)
Number of oocytes retrieved	11,06 ± 7,49
Number of mature oocyte	8,46 ± 6,54
Maturation rate (%)	75,0(40-100)
Fertilization rate (%)	65,2 ±29,3
GDF-9 follicular fluid (pg/dl)	163,0(48,0-537)
GDF 9 serum (pg/ml)	260,63±121,82
BMP-15 follicular fluid(pg. ml)	58,30±31,54
BMP-15 serum (pg/ml).	74,0(610,0-1,0)

There were abnormally distributed data (Shapiro-wilk) test for normality, P<0.05) for GDF-9 (follicular fluid), BMP-15 (serum), maturation rate and age; and there were normally distributed for GDF-9 (serum), BMP-15 (follicular fluid) and fertilization rate.

Table 2 shows the prediction of GDF-9 and BMP-15 follicular fluid levels, GDF-9 and BMP-15 serum levels as the oocyte quality parameter. There was no significant correlation between GDF-9 serum and FF (P>0,05) but there was a significant correlation BMP-15 serum and follicular fluid (P<0,05).

After a bivariat analysis of the data with linear regression, there was a correlation between BMP-15 serum–FF, GDF-9 serum and BMI with maturation rate. While there was a correlation between GDF-9 serum, BMP-15 serum–FF and age with fertilization rate. The result from correlation analysis were given in table 4.

Table 2: Correlation of GDF-9 and BMP-15 in follicular fluid and serum

	GDF-9 FF	BMP-15 FF
GDF-9 serum	p=0,245 r=0,219	
BMP-15 serum		p=0,001 r=0,568

P = significance. r = correlation coefficient

Table 3 showed the correlation of GDF-9 serum and FF and BMP-15 serum and FF with maturation and fertilization rate.

Table 3: The correlation of gdf-9 in serum and gdf-9 in follicular fluid with the rate of maturation and the rate of fertilization as well as the correlation of bmp-15 in serum and bmp-15 in follicular fluid with the rate of maturation and the rate of fertilization

	Maturation rate(sig.)	Fertilization rate (sig.)
GDF-9 FF	p = 0,321. r=0,188	p = 0,307 r= 0,193
GDF-9 serum	p = 0,336 r=0,182	p = 0,633. r= 0,091
BMP-15 FF	p = 0,415. r=0,155	p = 0,365. r= 0,172
BMP-15 serum	p = 0,001 r=-0,577	p = 0,140. r= 0,276

P= significance. r = correlation coefficient

After a bivariate analysis of the data with linier regression, there was correlation between BMP-15 serum–FF, GDF-9 serum and BMI with maturation rate. While there was a correlation between GDF-9 serum, BMP-15 serum–FF and age with fertilization rate. The result from correlation analysis were given in table 4.

Table 4: Multivariate analysis between maturation rates, fertilization rates and BMP-15 serum, age dan BMI

	Maturation rates			Fertilization rates		
	ß	CI 95%	p	ß	CI 95%	p
BMP-15 fol	0,001	(0,002-0,004)	0,489	0,008	(-0,004-0,004)	0,154
BMP-15 serum	0,001	(-0,002-0,000)	0,007	0,001	(0,000-0,002)	0,119
GDF-9 fol	0,000	(0,000-0,001)	0,119	0,001	(0,000-0,001)	0,125
GDF-9 serum	0,000	(-0,001-0,000)	0,283	0,001	(0,000-0,001)	0,167
Age	0,013	(-0,034-0,008)	0,202	-0,031	(-,060-0,003)	0,031
BMI	0,002	(0,020-0,024)	0,868	0,012	(0,018-0,042)	0,401

CI 95%: confidential interval, P: significance, ß: beta

DISCUSSION

The aim of this research is to develop and measure GDF-9 and BMP-15 in follicular fluid and serum analysis and diagnostic potential in reproductive medicine. According previous studies it is known that as oocyte-secreted factors, GDF-9 and BMP-15 concentrations were very essential for fertility including in humans, where both of these factors are considered as factors with autocrine or paracrine secreted oocyte which acts in the ovarian follicles to regulates the function of cells around the oocyte were granulosa cells and cumulus cells as folliculogenesis and fecundity regulators [6, 12]. GDF-9 and BMP-15 have homology in high degrees of amino acid and protein structure, seem at an overview of expression and function and also the interaction of both. These factors were a potent regulator of granulosa cells and cumulus cells function to improve the quality of oocyte. GDF-9 has the effect of anti-apoptosis on the stage of preantral and early follicle, stimulates the proliferation of granulosa cells. BMP-15 also has the ability to increase the proliferation of granulosa cells and very effective as anti-apoptosis in cumulus cells, stimulates the expression of several other proteins such as EGF, which esensial for the expansion of cumulus cells. It certainly plays an essential role on the stage of cell proliferation, apoptosis process, luteinization, and expansion of the cumulus cells metabolism [6, 12, 34–38].

Based on the role of both these factors on the quality of oocytes (maturation rate and fertilization rate) our study want to see whether the concentration of both these factors can be used a marker of quality of the oocyte. In our study the distribution of GDF-9 and BMP-15 were detected on both follicular fluid and serum, whe on the previous study it is known that GDF-9 and BMP-15 concentration levels were low in serum even only detected in 29% of subjects (GDF-9) and 61%(BMP-15).[6] But the results of our study were surprising because have a high variation and the concentration levels in the serum were higher than in the follicular fluids. This may be a result of follicular fluid sampling that was conducted by pooling, which caused the results obtained to look smaller than serum due to dilution process, but some of the previous studies that take a single mature follicle also shows a high variation in the level of concentration of GDF-9 and BMP-15 in follicular fluid [36–38]. The other possibilities were reagen spesivity in KIT, indeed this reagent is only specific to examination of serum, plasma and body fluids, so no specific mention of the follicular fluid of the ovaries. Also, it could be possible the existence of cross-reactivity of monoclonal antibodies of the reagent with a substance wich analogous to the GDF-9 in the serum is higher compared to the cross reactivity in the follicular fluid wich is pure only binds to the GDF-9 [6]. It was also obtained in a study by Riepsamen et. al (2019) [6], obtained levels of GDF-9 were high in he srum of postmenopausal women, it was thought possible consequences there were other cell types that express GDF-9 and contributes to detection of the levels of this protein in peripheral serum such as the cells of the granulosa and cumulus cells, adrenal and other celss [6, 12, 46–49]. Fortunately, we can determine the concentration levels of all the samples from the serum, so than in the future the examination level of GDF-9 or BMP-15 follicular fluid can be replaced with serum examination. GDF-9 and BMP-15 serum does not appear to significantly decrease with age in women of reproductive age (20–40 y). Though as GDF-9 and BMP-15 are generally considered to be oocyte-specific products and the number fall with age. Although the magnitude of the variation in serum/follicular fluid GDF-9 and BMP-15 between individual subject, the concentration appears to be constant in the subject and are not affected by superovulation induction with gonadotropins. BMP-15 and GDF-9 may be present in serum must be freed from being tied to the granulosa/cumulus extra-cellular proteoglycan matrix and then transit in the follicular basal lamina then migrate to the theca extracellular matrix (ECM) to enter into blood vessel [13, 15, 34].

Distribution of BMP-15 in follicular fluid average 58,30 pq/ml±31,539 (normal distribution) and BMP-15 serum levels was 74,0 pq/ml(1,0–610,0) (abnormal distribution), it turns out that there was a positive correlation between the level of BMP-15 FF and BMP-15 serum (P = 0,001); thus it can be said that the concentration of BMP-15 serum was represented the concentration/levels of BMP-15 follicular fluid.

GDF-9 and BMP-15 are all factors secreted by the oocyte (OSF) is a very important factor in determining the quality of the oocyte, where these two factors are very instrumental in the process of follikulogenesis especially in the case of constant two-way communication between cells-cells that surround the oocyte with oocyte itself, both as a means of transfer of nutrients and signal paracrine, which of course largely determines the development of compartment follicular, maturation of the oocyte and the ability of the acquisition of the oocyte to fertilization. An oocyte prior to become an oocyte which is competent to achieve miosis complete (from prophase i until it's metaphase ii) and are capable for fertilization by a sperm to produce embryos that viabel, begins at the moment of antral follicles then the complex cumulus (COC) contribute to provide the benefits of metabolic and maintain a phase of miosis for the growth of the oocyte. at the same time oocyte secreted factor (OSF) mediate the metabolism, maturation and persistence of complex cumulus (COC). in some previous research, it is known that the levels of GDF-9 and BMP-15 are positively correlated to the rate of maturation and the rate of fertilization even the birth of life, which could be assumed as a representation of the quality of the oocyte [37–50].

The results from several previous study showed that GDF-9-BMP-15 concentration may be useful to predict the number of good oocyte quality in an IVF and also for detecting potential poor responders' patients. But in our study, there was no correlation between GDF-9 and BMP-15 follicular or serum with maturation rate or fertilization rate. However, in our study, from measurements of the levels of GDF-9 in serum, GDF-9 in follicular fluid bmp-15 in follicular showed no relationship/correlation to the maturation and fertilization rate, even obtained the existence of a negative correlation between bmp-15 serum with the maturation rate. Although the role of GDF-9 and BMP-15 *in vivo* are very good role since the preantral to the oocyte mature and its role in folliculogenesis, the development of the follicles, anti-apoptosis and the role of communication in two directions between the oocytes with the complex of cumulus (COC) but the possibility of other factors also need to be examined to determine the significantly affect the quality of oocytes, in this case seen mainly through the maturity of the oocyte. We did not find a direct relationship between individual FF or serum GDF-9 or BMP-15 level and oocyte developmental competence. In our study, individual FF or serum GDF-9 or BMP-15 concentration was quite variable, but it does not seem to reflect oocyte fertilisability or subsequent embryo quality; this non-association could partly be explained by our study samples, where FF was collected by pooling or FF was collected from fully matured follicle and the oocyte was already fully-matured [52, 57, 63].

BMP-15 is a marker of the quality of the oocyte, which affects the physiology and growth of follicles. In granulosa cells this protein also functions as a growth factor of in a functional oocytes regulation of proliferation and differentiation. In addition, it affects the development of the granulosa cells layer–oocytes (the cumulus layer), so its levels suggest have a positive correlation with maturation or fertilization rate, but from our result of BMP-15 serum that showed a negative correlation with maturation rate (coefficient correlation-0,577, P = 0.001), it is possible due to high variation of data and the limited sample number/quantities. Likewise, the other correlation from GDF-9 serum or FF and BMP-15 FF were showed no correlation with maturation rate and fertilization rate even though from previous research was suggest that GDF-9 and BMP-15 act synergistically to promote the ovulation of the oocyte surrounded by granulosa cells [55, 56].

From the review some other factors that we can see its influence by multivariate analysis, where the average age on the subject of this study was 35 y (26,0-39,0) and having performed a statistical test to see its effect on the rate of maturation it turns out that no correlations (p = 0,620), it is possible there are some other factors that affects to maturation rate. However, there is a negative correlation (correlation coefficient-0,386) between the age with the rate of fertilization (p = 0,035) so that it can be said that the lower fertilization rate on increased age, it was certainly in accordance with several other studies which stated that the age factor affects the level of fertilization [6, 13, 15, 41].

From the recent studies stated that to assess the quality of oocytes has been widely proposed to conduct the examination of biochemical molecules as a predictor, however, the morphology of the oocyte seems to still a method that is popular to determine oocyte competent [47]. Although there was in fact, the involvement of GDF-9 and BMP-15 in follikulogenesis, steroid genesis and maturation of oocytes, but the concentration of GDF-9 and BMP-15 in follicular fluid or serum individually cannot be used as a predictor of development or oocytes competent.

CONCLUSION

There were abnormal distribution and no correlation between levels of GDF-9 serum and follicular fluid. No correlations between levels of GDF-9 serum-FF and BMP-15-FF, and maturation rates and fertilization rates. There was negative correlation between BMP-15 serum and maturation rates. GDF-9 and BMP-15 serum and follicular fluid cannot predict oocyte quality.

This study indicates that GDF-9 and BMP-15 were not the only factor that affects to maturation rate and fertilization rate. It is recommended for further research to assess the factors that play a role of the development and quality of oocytes.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally.

CONFLICT OF INTERESTS

All authors have none to declare.

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