ORIGINAL ARTICLE:

Effect of bone marrow mesenchymal stem cells transplantation on BMP-15 expression and Graafian follicle count in mice model of endometriosis

Ratriana Via Parasti[1], Widjiati[2], Sri Ratna Dwiningsih*[1]
[1]Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Dr Soetomo Hospital, Surabaya, Indonesia, [2]Department of Veterinary Obstetrics, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia

ABSTRACT

Objectives: To determine the effect of bone marrow mesenchymal stem cells (BMSCs) on BMP-15 expression and Graafian follicle count in endometriosis mice.
Material and Methods: This study was a laboratory randomized clinical trial on Mus musculus. The object of the study was 42 mice which were divided into 3 groups, the control, endometriosis, and endometriosis + BMSCs groups. Comparison of BMP-15 expression and Graafian follicle count between groups was evaluated.
Results: Immunohistochemical analysis showed that BMP-15 expression in control, endometriosis, and endometriosis + BMSCs groups had p=0.551, p=0.446 and p=0.917 with ANOVA test p=0.273, indicating no statistically significant differences between groups . Graafian follicular count in the three groups had p=0.31, p=0.001, and p=0.006, with the Kruskal-Wallis test p=0.001. Graafian follicles in the endometriosis + BMSCs group were higher than those in control and endometriosis groups.
Conclusion: In the endometriosis mouse model with bone marrow stem cell transplantation the BMP-15 expression in each group did not show a difference, but a significant difference was found in the number of Graafian follicles.

Keywords: Endometriosis; bone marrow stem cells; BMP-15

ABSTRAK

Tujuan: Untuk mengetahui efek BMSCs terhadap ekspresi BMP-15 dan jumlah folikel de Graaf pada mencit model endometriosis
Bahan dan Metode: Penelitian ini merupakan randomized clinical trial eksperimental laboratorium terhadap Mus musculus. Objek penelitian adalah 42 mencit yang dibagi menjadi 3 kelompok, yaitu kontrol, endometriosis, dan endometriosis + kelompok BMSCs. Kemudian, perbandingan ekspresi BMP-15 dan jumlah folikel de Graaf antar kelompok dievaluasi.
Hasil: Analisis imunohistokimia menunjukkan bahwa ekspresi BMP-15 pada kelompok kontrol, endometriosis, dan kelompok endometriosis + BMSCs adalah memiliki p=0,551, p=0,446 dan p=0,917 dengan uji ANOVA p=0,273, menunjukkan tidak terdapat beda bermakna secara statistik antar kelompok. Jumlah folikel de Graaf pada ketiga kelompok memiliki p=0,31, p=0,001, dan p=0,006, dengan uji Kruskal-Wallis p=0,001. Folikel de Graaf pada kelompok endometriosis + BMSCs lebih tinggi daripada pada kelompok kontrol dan endometriosis.
Simpulan: Pada model tikus endometriosis dengan transplantasi sel punca sumsum tulang ekspresi BMP-15 pada tiap kelompok tidak menunjukkan perbedaan, namun perbedaan bermakna didapatkan pada jumlah folikel de Graaf.

Kata kunci: Endometriosis; sel punca sumsum tulang; BMP-15

***Correspondence:** Sri Ratna Dwiningsih, Department of Obstetrics and Gynecology, Faculty of Medicine, Universitas Airlangga, Dr. Soetomo Hospital, Jalan Prof dr Moestopo 6-8, Surabaya 60286, East Java, Indonesia. E-mail: ratna_hamzah@yahoo.com

pISSN:0854-0381 ● eISSN: 2598-1013 ● doi: http://dx.doi.org/10.20473/mog.V26I32018.107-111
● Maj Obs Gin. 2018;26:107-111 ● Received 10 Jan 2018 ● Accepted 16 May 2018

INTRODUCTION

Endometriosis is a disorder of the female reproductive system, where the endometrium grows outside the uterus. This condition is most often found in the ovaries and peritoneum, also causing premenstrual pain and dysmenorrhea. The main symptoms in this disease are pelvic pain, dysmenorrhea and dyspareunia. Endometriosis is one of the causes of infertility and is diagnosed in 25-40% of infertile women. Although it can interfere with fertility, the mechanism is still uncertain.[1]

Folliculogenesis is the center of female fertility and is formed by communication of the endocrine complex between the central nervous system, the hypothalamus and the ovary. In this case, the intra-ovarium paracrine message is the basis of oocyte maturation, growth and development of follicle, and provides adequate sensitivity for gonadotropin stimula-tion. Therefore, messages originating from oocytes should be continuous and can assemble a series of formations from the primordial follicles and control the forthcoming maturation of the follicles.[2] The decreased quality of oocytes may be a consequence of folliculo-genesis disorders. A study by Bahtiyar et al. even obtained follicular fluid in endometriosis patients that contained factors that can affect the pattern of endo-metrial growth in other tissues.[3]

BMP-15 is a growth and differentiation factor originating from oocytes. This factor is an important regulator in folliculogenesis and granulosa cell activity.[2] A recent study has shown rapid progress in the concept that oocytes have an important role in regulating the func-tion of surrounding somatic cells. Research by Pasquale et al. identify BMP-15 mutations in women who experience ovarian failure due to ovarian disgenesis.[4]

Levels of GDF 9 in female follicle endometriosis are found to be lower than in non-endometriosis women.[5] However, until now there has been no journal that mentions the existence of an association between BMP-15 expression levels and endometriosis. Endometriosis is also said to increase granulosa cell apoptosis so that it can affect the process of folliculogenesis and steroidogenesis.[6]

Stem cells are a source of cells that can renew themselves for a long time. Because it has the ability to become various types of cell, stem cells can be used for regenerative therapy in diseases caused by cell damage.[7,8] This study assumes that the administration of bone marrow mesenchymal stem cells can reduce granulosa cell apoptosis and increase BMP-15 expression so that paracrine communication between granulosa cells and oocytes is improved, thereby it can hold a folliculogenesis process.

This study observed the BMP-15 expression as an indicator of oocyte quality in endometriosis because studies using GDF 9, which is a homologous sequence of BMP-15 as an oocyte quality profile of endometriosis, had been carried out. This study aims to analyze differences in BMP-15 expression in endometriosis models as an indicator of oocyte quality after the administration of mesenchymal bone marrow stem cells.

MATERIALS AND METHODS

This study was a laboratory experimental study in mice using randomized double blind design. This study was conducted in August 2017 to September 2017 in in vitro Embryology Laboratory, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia. Study sample compris-ed 42 female mice (Mus musculus) that had met the inclusion criteria, aged 3 months, weight 20-40 g.

The subjects were divided into three groups randomly. The first group (K0), as a control, injected with 0.9% NaCl intraperitoneally. The second group (K1) was a group of mice with endometriosis models who were given with intramuscular (IM) injections of cyclosporine A and estrogen, then intraperitoneal (IP) injection was given. Three days later this group received the second estrogen injection, then eight days later received a MEM buffer injection intravenously (IV). The third group (K2) was a group of mice with endometriosis models who obtained injections of cyclosporine A and estrogen injections intramuscularly (IM), then intraperitoneal (IP) endometrial injection. Eleven days after the administration of endometrium this group received a transplant of 2×10^7 bone marrow mesenchymal stem cells intravenously (IV). All three groups were given ovarian stimulation with PMSG and hCG and then oocyte harvesting was carried out.

Before determining the type of statistics used, data normality test was conducted to determine the normality of data distribution. In this study, BMP-15 expression data and the number of Graafian follicles obtained from each study group were tested with a normality test with in a significance level of $p > 0.05$. If the distribution was normal ($p>0.05$), the One-Way ANOVA test was carried out because in this study there were more than two groups. If it was abnormally distributed ($p <0.05$), a non-parametric test was carried out, followed by a post-hoc test. Then, a regression test was performed to see the effect of some variables on other variables.

RESULTS AND DISCUSSION

Bone Morphogenetic Protein-15 (BMP-15)

Table 1. Mean expression of BMP-15 in the control, endometriosis, and endometriosis + BMSCs groups

	BMP-15
K0 (Control)	1.8±0.8
K1 (Endometriosis)	1.6±1.2
K2 (Endometriosis+BMSCs)	2.1±1.0

Table 1 shows mean BMP-15 expression in each group. The lowest mean BMP-15 was found in the endometriosis group, while the highest mean was found in endometriosis + BMSCs group. In the normality test using Shapiro-Wilk, the degree of significance of the control group, endo-metriosis, and endometriosis + BMSCs were p=0.551, p=0.446 and p = 0.917. Because the p value in 3 groups was >0.05, then the distribution of the three groups' data was normal. Because the data were normally distributed, the test was followed with parametric one-way ANOVA test.

Table 2. Comparison of BMP-15 expressions between groups (One-Way ANOVA test)

	n	Mean ± SD	P value
K0(Control)	11	1.8±0.8	0.50
K1(Endometriosis)	11	1.6±1.2	
K2 (Endometriosis+BMSCs)	11	2.1±1.0	

Table 2 shows the comparison of BMP-15 expression using one-way ANOVA test with a value of p=0.05, which means that there is no significant difference in BMP-15 expression between groups.

Graafian follicle count

This study compared the number of Graafian follicles between control, endometriosis, and endometriosis + BMSCs groups. The lowest Graafian follicle count was found in the endometriosis group, while the highest mean was obtained in the control group. The normality test using Shapiro-Wilk showed that the three groups had abnormal data distribution (p <0.05), so the test was continued using Kruskal-Wallis test. Table 3 with the Kruskal-Wallis test resulted in a value of p=0.001 (p<0.05), it can be concluded that there were at least differences in Graafian follicle count between 2 groups statistically, so that it was followed by Mann-Whitney post-hoc test.

Table 3. Comparison of Graafian follicles count between groups (Kruskal-Wallis test)

	N	Median (Min-Max)	P value
K0 (Control)	14	4(2-9)	0.001
K1 (Endometriosis)	14	1.5(1-5)	
K2 (Endometriosis+BMSCs)	14	3.5(2-8)	

Table 4. Mann-Whitney post-hoc analysis comparing Graafian follicle counts between groups (Kruskal-Wallis test)

	N	Median (Min-Max)	P value
K0 (Control)	14	4(2-9)	0.001
K1 (Endometriosis)	14	1.5(1-5)	
K2 (Endometriosis+BMSCs)	14	3.5(2-8)	

Table 4 shows a significant difference between endometriosis group and endometriosis + BMSCs group, also between control group and endometriosis group. However, no significant differences were found bet-ween control group and endometriosis + BMSCs group.

Characteristics of the samples

BMP-15 has an important role in the regulation of folliculogenesis and follicular function in the ovary. In vitro studies in mice used recombinant BMP-15 identified granulosa cells as the predominant target cell type 15 BMP in the ovary.[9] The presence of introvarian paracrine communication is important for follicular growth and maturation. Although the signal network between oocytes and the surrounding granulosa cells is not widely known, it is currently suspected that some members of the TGF-ß superfamily, for example, BMP-15 and GDF 9, have important roles. BMP-15 along with GDF 9 causes cumulus expansion by activating the expression of several genes, including hyaluronan synthase 2 (HAS 2), cyclooxygenase 2 (COX 2), and gremlin 1 (GREM 1).[6]

In this study the lowest BMP-15 expression was found in the endometriosis group, and the highest mean was found in the endometriosis + BMSCs group. However, when comparisons were made between groups, we did not find statistically significant differences. The expression of BMP-15 in the endometriosis group was the lowest among the groups. This indicates that the presence of a disorder in folliculogenesis can be seen from a decrease in BMP-15 expression. This is in accordance with the results of Sato et al. study which states that endome-triosis causes PI3K pathway dysregulation.[10]

This result was consistent with a study of Liu et al., who found that PI3K pathway disorder in oocytes causes infertility in mice with an increase in FoXo3a which directly decreases BMP-15 expression, which can then interfere with paracrine communication from granulosa, oocyte and theca cell cells, resulting in folliculogenesis disorder.[11] Otsuka's study also shows that BMP15 also plays a role in the regulation of cumulus cell apoptosis. In bovine ovary, the removal of granulosa cells from cumulus oocyte complex (COC) will trigger apoptosis. This apoptosis can be prevented by providing BMP-15.[12]

Follicle is a functional unit of the ovary and each follicle consists of oocytes surrounded by one or more somatic cell layers. In order for the function of steroidogenesis and ovulation to be realized, the follicle must develop through a series of highly coordinated stages of development.[13] Follicle development is a complex process involving signals between organ systems.[14]

Follicle develops through premordial, primary, and secondary stages before the antrum/cavity is formed in the follicle.[15] Primordial follicles are the basic reproductive units which present as dormant oocyte pools. Morphologically, the primordial follicle consists of a primary oocyte surrounded by flat-layer epithelium granulosa cells and basement membrane.[16] Along with the development of maturation, granulosa cells become cuboidal and these follicles are called primary follicles.[17] Development of secondary follicles begins with the presence of a second layer of granulosa cells. This involves changes in granulosa cells and the acquisition of theca cells. Then, the follicles develop into tertiary follicles. This phase is characterized by the formation of antrum/cavity in the follicle.[16]

In this study Graafian follicles count in the endometriosis group was the lowest among the groups. Then, a comparative test was conducted to find the level of significance between groups. The results showed a comparison of the average number of Graafian follicles per group, where the Graafian follicles count in endometriosis group differed significantly from that in the endometriosis + BMSCs group. This showed that bone marrow mesenchymal stem cell transplantation improved somatic environment of the ovary, as characterized by improvement in folliculogenesis.

Lee et al stated that administration of bone marrow stem cells activates oogenesis directly or indirectly. Directly, stem cells activate the inhibited cell cycle, and indirectly these cells stimulate the microenvironment of the damaged cell.[18] The administration of stem cells can also affect local stem cells that already exist in the ovary or stem cells outside the ovary to homing to the ovary, thereby improving the micro-environment in the ovarian follicle area.[19]

Hendarto et al., in their study on the ovaries of mice that received cisplatin, found that folliculogenesis was lower compared to that of the controls. Cisplatin will destroy oocytes and granulosa cells and disrupt the secretion of growth factors. The result of this disorder is the reduction in follicular growth in the group receiving cisplatin. The administration of bone marrow transplantation has succeeded in repairing oocytes and granulosa cells and the production of growth factors, with the final result of the folliculogenesis improvement. This folliculogenesis repair is the basis for further studies regarding the mechanism of mesenchymal bone marrow stem cells in improving folliculogenesis.[20]

A subsequent study by Santoso, who provided rat bone marrow stem cell therapy in polycystic ovary syndrome models to observe folliculogenesis, found that folliculogenesis examination using ovarian Haema-toxillin eosin staining showed folliculogenesis improve-ment in the treatment group, which was characterized by improved follicle formation from the primary to Graafian follicles.[21]

This study proves that bone marrow mesenchymal stem cells will be homing towards ovarian follicles damaged by endometriosis and subsequently normalize the somatic environment in the area. This will improve ovarian cellular communication. Furthermore, folliculogenesis starts normally again, which is indicated by the occurrence of growth process and the increase the number of Graafian follicles count.

CONCLUSION

Endometriosis mice model with bone marrow stem cell transplant showed no difference in BMP-15 expression in each group, but revealed significant differences in de Graaf follicles.

REFERENCES

1. Xu B, Guo N, Shi W, et al. Oocyte quality is decreased in women with minimal or mild endometriosis. Sci Rep. 2015; 5: 10779.
2. Persani L, Rosetti R, Di Pasquale E, et al. The fundamental role of bone morphogenetic protein 15 in ovarian function and its involvement in female fertility disorders. Hum Reprod Update. 2014;20(6) :869-83
3. Bahtiyar MO, Seli E, Senturk LM, et al. 1998. Follicular fluid of women with endometriosis

stimulates the proliferation of endometrial stromal cells.Human Reproduction. 1998;13(12):3492-5
4. DiPasquale E, Beck-Peccoz P, Perani L.. Hyper-agonadotropic ovarian failure associated with inherited mutation of human bone morphogenetic protein-15 (BMP-15) gene. Am J Hum Genet. 2004;75 (1):106-11.
5. Hendarto H, Profil TNF-, GDF-9 dan Hyalronan pada gangguan folikulogenesis sebagai gambaran penurunan kualitas oosit pasien infertil dengan endometriosis. Dissertation. Universitas Airlangga; 2007.
6. Sanchez, AM, Somigliana E, Vercelini P, et al. Endometriosis as a detrimental condition for granulosa cell steroidogenesis and development; From molecular alterations to clinical impact. Journal of Steroid Biochemistry and Molecular Biology, 2016;155:35-46.
7. Lindvall, O. & Kokaia, Z.,2005. Stem cell therapy for human brain disorders. Kidney Int, 68, 1937-9.
8. Watt FM, Driskell RR. The therapeutic potential of stem cells. Philos Trans R Soc Lond B Biol Sci. 2010;365:155-63.
9. Moore RK, Shimasaki S. ,2002. Molecular biology and physiological role of the oocyte factor, BMP-15. Molecular and Cellular Endocrinology 2005; 234(1-2):67-73
10. Sato N, Tsunoda H, Nishida M, et al. Loss of heterozygosity on 10q23.3 and mutation of the tumor suppressor gene PTEN in benign endometrial cyst of the ovary: possible sequence progression from benign endometrial cyst to endometrioid carcinoma and clear cell carcinoma of the ovary. Cancer Res. 2000;60:7052–6.
11. Liu K, Rajareddy S, Liu L, et al. Control of mammalian oocyte growth and early follicular development by the oocyte PI3 kinase pathway: New roles for an old timer. Dev Biol. 2006;299(1):1-11.
12. Otsuka F, McTavish KJ, Shimasaki S. Integral role of GDF 9 and BMP-15 in ovarian function. Molecu-le Reproduction and Development. 2011; 78:9-21.
13. Knight PG, Glister C.,2006. TGF-beta superfamily members and ovarian follicle development. Reproduction 2006;132:191-206.
14. Rajkovic A, Pangas SA, Matzuk MM. Follicular development: Mouse, sheep, and human models. In: Plant TM, Zeleznik AJ, editors Knobil and Neill's physiology of reproduction. 4th edn. London: Elsevier; 2006. p. 947-95.
15. McGee EA, Hsueh AJ 2000. Initial and cyclic recruitment of ovarian follicles. Endocr Rev. 2000; 21:200-14.
16. Rosen MP, Johnstone E, Addauan-Andersen C, Cedars MI. A lower antral follicle count is associated with infertility. Fertil Steril. 2011;95(6) :1950-4
17. Leslie P. Gartner, James L. Hiatt. Color atlas and text of histology. Lippincott Williams & Wilkins, 2012
18. Lee HJK, Selesmiami Y, Niikura T, et al. Bone marrow transplantation generates immature oocytes and rescues long term fertility in a preclinical mouse model of chemotherapy indued premature ovarian failure. J Clin Oncol. 2007 Aug 1;25(22) :3198-204.
19. Tilly JL, Johnson J. Recent arguments against germ cell renewal in the adult human ovary: Is an absence of marker gene expression really acceptable evidence of an absence of oogenesis? Cell Cycle. 2007;6(8):879-83.
20. Hendarto H, Komarhadi M, Darmawanti E, Widjiati, S The effect of bone marrow transplantation on oocyte-granulosa cell interaction and follicular development of cisplatin-induced ovarian failure in rat. J Stem Cell Res Ther. 2013:3;1.
21. Santoso B, Sulistiyono A Efektivitas terapi rat bone marrow stem cell pada tikus model sindroma ovarium poli kistik terhadap folikulogenesis, ekspresi Transforming Growth Faktor B. Penelitian Unggulan Perguruan Tinggi. Universitas Airlangga 2014.