The Effect of Adiponectin Recombinant in Rattus Norvegicus with Polycystic Ovarium Syndrome Model on Anti-Müellerian Hormone Expression

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Abstract

Background: Polycystic Ovary Syndrome (PCOS) is an important disease that causes various health problems in women. Ovulation - infertility disorders are found in 60-85% woman with PCOS. Anti-Muellerian Hormone (AMH), a protein from Transforming Growth-b Family, often increased in patient with PCOS and associated with severity and poor prognosis for assisted reproductive outcome. Dietary and lifestyle changes are the first-line therapies but consistent application of this method is difficult to attain for people with PCOS. Adiponectin, a product by adipose cells have a significant reduction in obese and women with PCOS. The purpose of this study was to see the differences in AMH expression between mice with polycystic ovary model treated with adiponectin recombinant and mice with polycystic ovary model treated with placebo and control.

Method: This research is an experimental study with a Post Test Only Control Group design. The sample of this study was 3 months female Rattus norvegicus Wistar strain weighing 110-120 g. The sample size was 36, which were divided into three groups, K0 (control group of mice with polycystic ovary model), K1 (group of mice with polycystic ovary model that received placebo injection), and K2 (group of mice with polycystic ovary model that received 5 µg/ml injection of recombinant adiponectin). Polycystic ovary model made by injecting 10 mg/Kg body weight testosterone propionate for 14 days. The injection is carried out at the proestrous stage. The three groups of mice in a period of 3 weeks after treatment, were surgically removed according to the sampling procedure, then the ovaries were given a code and immunohistochemical staining was performed to see the AMH expression in the ovaries. The data were collected, then statistical analysis was carried out using SPSS software.

Result: The results showed that the AMH expression value in the K0 group was 3.3 (± 0.86), K1 3.1 (± 0.77) and K2 3.4 (± 0.81). The minimum score of the AMH expression for the K0 group is 2.2; with a maximum score is 5.1. For K1 group, the minimum score is 2,3 with a maximum score is 4.6. And for the K2 group, the minimum score of AMH expression is 2.2 and the maximum value is 4.6. From the results obtained, the homogeneity test was carried out with the Saphiro-Wilk test and showed

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that the data were normally distributed (control group p = 0.364; placebo group p = 0.09; adiponectin group p = 0.461). We then performed one-way ANOVA parametric test and found that the results of AMH expression in each group did not have a significant difference with a value of p = 0.651 (p> 0.05).

Conclusion: From the results above we conclude that in polycystic ovary model mice, there was no significant difference in AMH expression in mice treated with recombinant adiponectin compared to control and placebo group. Further research is needed to study the effect of adiponectin in pathogenesis of PCOS.

Keywords *: Polycystic Ovarian Syndrome, Anti-Müellerian Hormone, Adiponectin.*

Introduction

Polycystic Ovary Syndrome (PCOS) is an important disease that causes various health problems in women. Bozdak (2016), through a global study, found that PCOS occurs in 6-10% of the female population[1]. Obesity is found in 75% of cases of PCOS, and insulin resistance is more often found in cases of PCOS with obesity (70-80%) than those without obesity (25%). In PCOS, ovulation - infertility disorders are found in 60-85%[2]. Anti-Muellerian Hormone (AMH), a protein from Transforming Growth-b Family, often increased in patient with PCOS and associated with severity and poor prognosis for assisted reproductive outcome[3,4,5,6]. Diet and lifestyle changes are the first-line modalities therapies, unfortunately this method is difficult to attain for people with PCOS. Adiponectin, a product by adipose cells, is a homotrimer protein. The synthesis and secretion of these proteins mainly expressed during adipogenesis and associated with differentiation and decreased levels of lipids. A significant reduction in adiponectin secretion was found in obese and women with PCOS[5,6,7,8,9]. The purpose of this study was to see the differences in AMH expression between mice with polycystic ovary model treated with adiponectin recombinant and mice with polycystic ovary model treated with placebo and control.

Method

This research is an experimental study with a Post Test Only Control Group design. The sample of this study was 3 months female Rattus norvegicus Wistar strain weighing 110-120 g, with the exclusion criteria that had been used previously as experimental animals in other studies. The sample size was 36, which were divided into three groups, K0 (control group of mice with polycystic ovary model), K1 (group of mice with polycystic ovary model that received placebo injection), and K2 (group of mice with polycystic ovary model that received 5 µg/ml injection of recombinant adiponectin). Polycystic ovary model based on previous study was made by injecting the testosterone propionate with dose of 10 mg / KgBW for 14 days. The injection is carried out at the proestrous stage. The three groups of mice in a period of 3 weeks after treatment, were surgically removed according to the sampling procedure, then the ovaries were given a code and immunohistochemical staining was performed to see the AMH expression in the ovaries. The data were collected, then statistical analysis was carried out using SPSS software. Ethical eligibility was obtained from the Animal Care and Use Committee (ACUC), Faculty of Medicine, Airlangga University, Surabaya.

Result

The results showed that the AMH expression value in the K0 group was 3.3 (± 0.86), K1 3.1 (± 0.77) and K2 3.4 (± 0.81). The minimum score of the AMH expression for the K0 group is

2.2; with a maximum score is 5.1. For K1 group, the minimum score is 2,3 with a maximum score is

4.6. And for the K2 group, the minimum score of AMH expression is 2.2 and the maximum value is 4.6 (table 1 and figure 1). From the results obtained, the homogeneity test was carried out with the Saphiro-Wilk test and showed that the data were normally distributed

(control group p = 0.364; placebo group p = 0.09; adiponectin group p = 0.461). We then performed one-way ANOVA parametric test and found that the results of AMH expression in each group did not have a significant difference with a value of p = 0.651 (p> 0.05). The picture of AMH staining can be seen in figure 1 below.

Table 1. Descriptive value for AMH expression in each group

Group	AMH Score	Minimum	Maximum	95 % Confidence Interval		p
				Lower limit	Upper limit	
K0	3,3 ± 0,86	2,2	5,1	2,75	3,85	0,364
K1	3,1 ± 0,77	2,3	4,6	2,63	3,60	0,09
K2	3,4 ± 0,81	2,2	4,6	2,91	3,55	0,46

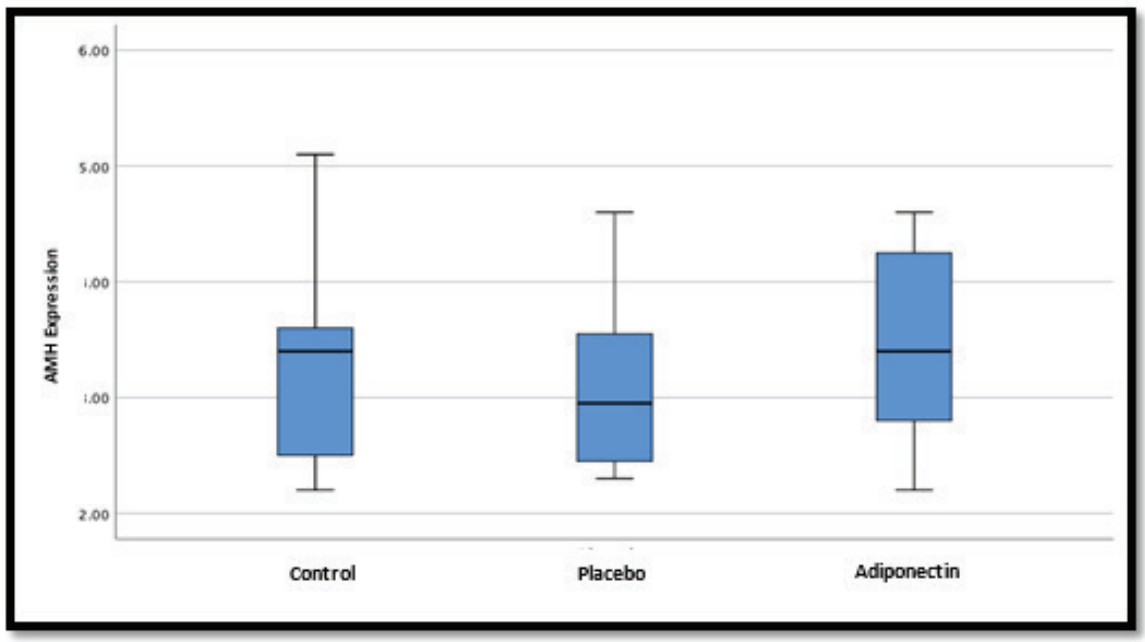

Figure 1. AMH expression score data in each group.

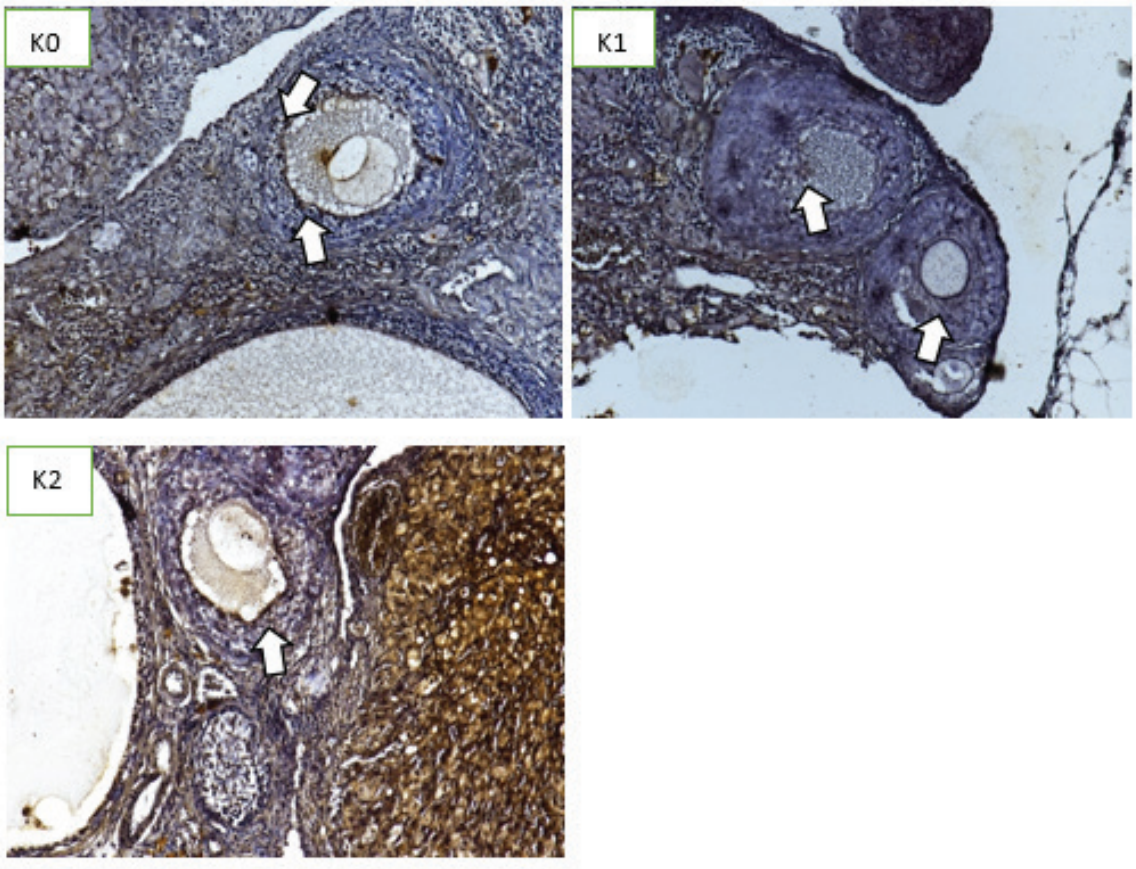

Figure 2. Immunohistochemical staining pattern for AMH in each group. AMH expression on each group showed with brown staining on granulosa cell (white arrow).

Discussion

The results of our study showed that there was no significant difference in AMH expression between mice with polycystic ovary model treated with adiponectin recombinant versus mice

with polycystic ovary control and placebo group. These results indicate that the administration of adiponectin in the polycystic ovary model, does not directly act on AMH expression. One of the possible mechanisms in explaining this phenomenon is that the change in PCOS, especially AMH in relation to folliculogenesis is complex with various hormonal and metabolic

mechanisms involved in it. The possible mechanisms involved are obesity and insulin resistance which play a major role in the pathogenesis of PCOS. Insulin resistance and increased levels of free fatty acids in obese patients with PCOS are one of the mechanisms that

cause this disease. The mechanism of obesity's effect on reproductive disorders is complex, but hyperinsulinemia with/without insulin resistance is thought to play a major role. Adiponectin can improve insulin resistance and cause a decrease in fatty acids in obesity[9,10,11,12,13]. Administration of adiponectin in previous studies will increase tissue sensitivity to insulin, reduce levels of free fatty acids, reduce LH secretion by the pituitary, reduce local production of androstenedione by theca cells and decrease the expression of LH receptors on theca cells all of which will result in decreased intrafollicular androgen production[13,14]. This decrease in androgen production will improve follicle growth thus decreased AMH

production. One of the drawbacks in our study is that in our experimental animals, obesity and insulin resistance were not found as metabolic factors for PCOS, so we could not determine the relationship between adiponectin and these factors on AMH expression. Administration of testosterone propionate for 14 days will result in polycystic ovary conditions. Research conducted by Muttaqin in 2009 shows that insulin resistance in mice is obtained after 28 days of testosterone propionate administration[16]. Based on our study, solitary hyperandrogenism condition without any underlying metabolic conditions e.g. insulin resistance or obesity did not produce significant changes in AMH expression. Another possible cause for the absence of differences in AMH expression in our study is the AMH polymorphism. Kaveenar (2007) showed that polymorphisms of genes responsible for AMH and AMH receptors coding affect AMH expression patterns in a person, especially those with PCOS[17]. It is currently unknown whether it is also found in mice, as from our study this polycystic ovarium model cannot induced this polymorphism, hence it is possible that no difference in AMH expression in the ovaries of the polycystic mouse model after administration of recombinant adiponectin in our study due to this factor. From the results of this study, we suspect that adiponectin does not result in changes in AMH expression via the hyperandrogenic pathway but possibly through the interaction between adiponectin and other factors, especially insulin resistance and obesity. From the results above we conclude that in polycystic ovary model mice, there was no significant difference in AMH expression in mice treated with recombinant adiponectin compared to control and placebo group.

Conclusion

From the results above we conclude that in polycystic ovary model mice, there was no significant difference in AMH expression in mice treated with recombinant adiponectin compared to control and placebo group. Further research is needed to study see the effect of adiponectin pathogenesis of PCOS.

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Ethical Clearance: This study was approved by *Animal Care and Use Committee* (ACUC) Veterinary Medicine Universitas Airlangga Surabaya.

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Conflict of Interest: -.

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