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Administration of *gallus domesticus* boiled chicken white egg increased amh levels of premenopausal female wistar rats

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ABSTRACT

Introduction: Aging is a complex multifactorial process and leads to functional changes one of which is the decline in reproductive function. Decrease in reproductive function in women in in the form of decreased ovarian reserve can be detected by low levels of Anti_mullerian Hormone (AMH). This study aims to prove the effect of administration of *Gallus domesticus* boiled chicken white egg increased AMH levels of premenopausal female wistar rats.

Methods: This research was experimental animal with Pretest-Posttest Control Group Design. The study conducted on 16 premenopausal female wistar mices, aged 17-18 months, weighing 250-260 grams. The mice were divided into 2 goups: the control group (placebo), which was given gelatin flour by 7.3 gram 3 times daily for 4 weeks and the group treted with *Gallus domesticus* boiled chicken white egg by 7.3 gram 3 times daily for 4 weeks by oral. Blood AMH level were measured before and after treatment for both group.

Results: The result showed that before treatment, mean AMH level both in control group and treatment group were not significantly different ($p > 0,05$). After treatment, mean AMH level both in control group and treatment group were significantly different ($p < 0,05$). The administration of *Gallus domesticus* boiled chicken white egg increased AMH levels by 0.804 ng/ml.

Conclusion: It can be concluded that administration of *Gallus domesticus* boiled chicken white egg increased AMH level. The mean AMH level after treatment had a significant difference between control group (placebo) and treatment group with $p = 0.001$.

Keywords: Anti-Mullerian Hormone, Aging, *Gallus domesticus* boiled chicken white egg

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INTRODUCTION

Every creature in this world will definitely experience the aging process. Physiologically, aging will cause changes in function and structure at both the cellular level, the organ and the body system. Aging is not only caused by the age factor but also influenced by various other factors such as internal factors such as free radicals, hormone deficiency, glycosylation process, methylation, apoptosis, decreased immune system and genes, as well as external factors such as unhealthy lifestyles, healthy, bad habits, environmental pollution, stress and poverty[1].

Aging can be known by seeing signs or changes that occur that can not only be seen physically but also anatomically, biochemically and molecularly. The signs of aging are also closely related to the function of various organs of the human body in supporting daily life[1]. One of the aging processes women will face is a gradual decrease in estrogen and progresterone hormones that cause aging of the reproductive organs as well as the weakening of ovarian function. At this time, the ovaries are no longer sensitive to stimuli from the brain, so the eggs cannot grow until they ripen so rarely ovulate and eventually stop.

Today, many women who experience early menopause and aging reproductive organs before the age that should be. Various researchers suggest that early menopause occurs in productive women of less than 40 years of age[2,3]. Based on the report of the Association of Endocrinology of Reproduction and Fertility of Indonesia (HIFERI) and In Vitro Indonesia Fertilization Association (PERFITRI) (2013), the percentage of female infertility in Indonesia indicates that many Indonesian women are experiencing aging problems in reproductive organs, thus experiencing infertility problems especially at young age[4]. Meanwhile, fertility is a vital aspect for women in carrying out their life. Therefore, it is important to make efforts to prevent,

slow down and overcome the aging by developing materials that can slow the aging process.

The most important predictors of ovarian response to hormone stimulation are age, biochemical parameters such as basal FSH levels in the early follicular phase, Anti-Mullerian hormone (AMH), and morphological characteristics such as the number of antral follicles (AFC) and ovarian volume. AMH is considered a more specific marker for ovarian responses to gonadotropin than other ovarian reserve assessments because AMH levels are stable throughout the menstrual cycle so that they can be measured in daily cycles and not affected by other hormonal variations, including use of oral contraceptives[5]. AMH values also have greater sensitivity than the value of inhibin B, FSH and estradiol in predicting ovarian follicle reserves and decreased AMH concentrations are reliable markers for evaluation of ovarian damage[6]. AMH concentrations can be a direct indicator of reduced ovarian function so that AMH measurements can play an important role in the diagnosis of patients with low ovarian reserves[7]. The decrease in ovarian reserve is indicated by low AMH concentrations.
Basically, low AMH concentrations in women are associated with decreased ovarian reserves. The decline in ovarian reserves due to aging occurs due to the decrease in growth hormone (GH) and insulin-1 growth factor (IGF-1) where growth hormone can increase the development of a number of antral follicles to the gonadotrophin-dependent stage and stimulate oocyte maturation, whereas IGF-1 increases granulosa cell proliferation, growth of steroidogenesis and oocytes in most mammalian species[8]. Thus, one way that can be done to increase ovarian reserve or AMH concentration is to increase GH.

Amino acids are a source of GH so that high intake of protein can stimulate GH secretion[9]. Higher protein intake increases ovarian activity by altering the IGF-I system by increasing follicular sensitivity to FSH and regulating oocyte quality[10]. Gorna et al. (2016) suggests that the amount and type of protein consumed may affect fertility and ovarian function of women because the cells present in the ovaries contain IGF-1 receptors and IGF-1 levels correlate with protein intake[11]. Thus, high protein administration may increase GH, thereby increasing folliculogenesis and ovarian reserve as indicated by increased AMH.

This study examines the protein intake of *Gallus domesticus* boiled chicken white egg. *Gallus domesticus* chicken white egg have a composition of amino acids more complete than amino acids other protein sources[12]. The protein content of *Gallus domesticus* chicken white egg is bigger than ras chicken egg[13].

The white part of the chicken egg is dominated by protein content and there is no fat content in it, whereas in the yolk part besides containing protein also contains trans-fat. Trans-fats can interfere with reproductive function by increasing insensitivity and inflammation of insulin in the body[14]. Therefore, the use of *Gallus domesticus* boiled chicken white egg can be a consideration for the community as a food intake to increase blood AMH so as to improve female fertility, ovarian function and can slow and delay the process of menopause. In this study will be used animals try white rats (Rattus Norvegicus) premenopausal females.

MATERIAL AND METODE

This research was experimental animal with Pretest-Posttest Control Group Design. The study conducted on 16 premenopausal female wistar mices, aged 17-18 months, weighing 250-260 grams. The mice were divided into 2 goups: the control group (placebo), which was given gelatin flour by 7.3 gram 3 times daily for 4 weeks and the group treted with *Gallus domesticus* boiled chicken white egg by 7.3 gram 3 times daily for 4 weeks by oral. Blood AMH level were measured before and after treatment for both group. Data were analyzed with paired t test and independent t test.

RESULT

Comparability test results with independent t-test average blood AMH levels after treatment in the control group with the treatment group were presented in Table 1.

Table 1. Mean Difference of Blood AMH Level After Treatment in Control Group with Treatment Group

Posttest Group	Mean of AMH (ng/ml)	SD	*t*	p
Control	0,337	0,046	-20,404	0,000
Treatment	1,177	0,107		

Table 1 showed that the mean AMH levels of blood after treatment (posttest) of the control group given placebo were 0.337 ± 0.046 ng/ml and the mean AMH levels of blood after treatment (posttest) treatment group were given *Gallus domesticus* boiled chicken white egg of 1,177 ± 0.107 ng / ml. Analysis of significance with independent t test

Table 2. Analysis of Treatment Effects

Posttest group	Mean dan SD AMH (ng/ml)		*t*	p
	Pretest	Posttest		
Control	0,364 ± 0,043	0,337 ± 0,046	7,825	0,000
Treatment	0,373 ± 0,044	1,177 ±0,107	-24,369	0,000

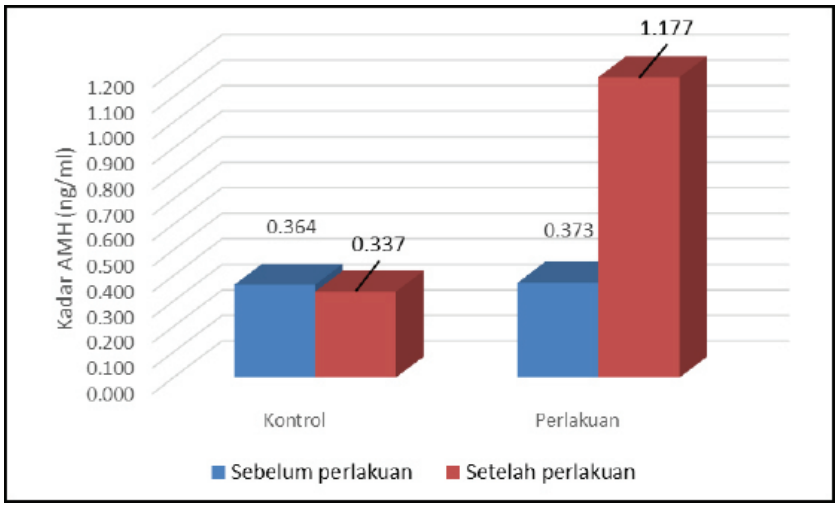

Figure 1. Average Comparison of AMH Blood Level Before and After Treatment in the Control Group and Treatment Group

obtained t value of -20.404 and p value of 0.000. These results indicate that the mean blood levels of AMH in the posttest between the control group and the treatment group had a significant difference (p <0.05).

The treatment effect analysis aims to compare the mean AMH levels between before and after treatment in each control group and treatment group. Results of significance analysis were tested with mean AMH levels tested with t-test paired and the results are presented in Table 2 and Figure 1.

Table 2 and Figure 1 showed that the mean of AMH blood was significantly lower than 0.364 ± 0.043 ng / ml to 0.337 ± 0.046 ng / ml after administering gelatin flour as much as 7.3 grams three times daily for 4 weeks (p <0.05). Significant increase in blood glucose level, ie, from 0.373 ± 0.044 ng / ml to 0.17 ± 0.107 ng / ml, was given *Gallus domesticus* boiled chicken white egg for 7.3 grams three times a day for 4 weeks (p <0 , 05).

DISCUSSION

These results suggest that mice that have been given boiled egg whites have significantly different AMH levels with mice AMH levels given gelatin flour where AMH rats are given *Gallus domesticus* boiled chicken white egg higher than the placebo AMH levels fed gelatin meal).

Gallus domesticus boiled chicken white egg have high protein content and are expressed by various researchers that the village chicken eggs have higher protein content than other poultry eggs. Bakhtra et al. (2016) stated that *Gallus domesticus* chicken egg have higher protein content than chicken eggs, duck eggs, and quail eggs[15]. Similar results were stated by Mawaddah (2010) in his study that the *Gallus domesticus* chicken white eggs have higher protein content than chicken eggs and duck eggs[16]. Mustika et al. (2014) in his research also reports that the protein content of *Gallus domesticus* chicken white egg is larger than the ras chicken eggs[13]. In addition to the high protein content, the *Gallus domesticus* chicken egg also has a complete and higher amino acid composition compared to the other amino acid composition of other protein sources[12]. Mawaddah (2010) states that the cooking process affects the protein content of eggs where the boiled eggs contain higher protein content than the steamed eggs[16].

This study only uses the white part of the chicken egg because the white part of the poultry eggs is dominated by protein content and there is no fat content. In the egg yolks other than containing proteins also contain trans fats where trans fats can interfere with reproductive function by increasing insensitivity and inflammation of insulin in the body[14]. Provision of *Gallus domesticus* boiled chicken white egg in premenopausal female wistar rats as much as 7.3 grams three times daily for 4 weeks can increase blood AMH levels by 0.804 ng/ml but the value of elevated AMH levels cannot be expressed as an increase in optimum AMH levels in terms of time because measuring AMH levels is only done for 4 weeks. Egg whites are an amino acid where amino acids are a source of growth hormone (GH)[9]. Growth hormone (GH) increases the development of a number of antral follicles to the gonadotrophin-dependent stage and stimulates oocyte maturation[8]. In addition, boiled egg white eggs have high protein content.

Protein intake was expressed as correlated with IGF-1 levels. The IGF-1 receptor is contained in the ovary cells so that the amount and type of protein consumed can affect fertility and ovarian function[11]. Higher protein intake increases ovarian activity by altering the IGF-I system by increasing follicular sensitivity to FSH and regulating oocyte quality[10]. High protein food intake is also stated to be used

as an anti-aging diet[14]. This is because the aging process occurs due to reduced GH secretion and circulation of IGF-1, and by providing high intake of protein especially animal protein can stimulate GH secretion and improve circulation of IGF-1, so a high intake of protein, especially animal protein can inhibit aging[8,9,10].

The results of this study indicate that along with increasing age, the aging process will occur. In women aged 35-45 years, physiologically will experience the aging process of reproductive function in the form of decreased ovarian function. At that age, women are at the premenopausal stage where there is a decrease in ovarian function due to decreased androgen hormone and growth hormone. These conditions make the reserve of ovaries decreased and the fertility of women decreased. Low ovarian reserve is characterized by low blood AMH levels. By giving a high intake of animal protein derived from egg whites boiled chicken can be said to increase blood levels of AMH. Thus, the intake of egg white boiled chicken is a diet high in animal protein that can be used for anti-aging diet that can inhibit the aging process, especially aging in the reproductive function. Through a diet high in animal protein intake, women can slow the aging of the reproductive function and prolong the process of aging by increasing blood levels of AMH. This is because the higher the level of blood AMH then the longer women experience menopause. This is as stated by Freeman et al. (2012) in his study that AMH levels <0.2 ng / ml occur on average 5.99 years before menopause in women aged 45-48 years and 9.94 years in women aged 35-39 years[17]. Regarding the AMH value of more than 1.5 ng / ml, menopause occurs on average after 6.23 years in the older group and after more than 13 years in the younger group.

Determination of blood AMH levels can serve as a good marker in assessing women's reproductive age compared with chronological age. This is because the aging process not only occurs due to the age factor, but is influenced by various other factors such as free radicals, reduced hormones, glycosylation process, methylation, apoptosis, decreased immune system, genes, unhealthy lifestyles, unhealthy diet, bad, environmental pollution, stress and poverty[1]. These conditions make some women may experience premenopausal conditions at younger ages, less than 40 years or known as premenopausal early. Therefore, by measuring blood AMH levels can serve as an early marker of the aging process of female reproductive function and by giving the intake of egg white boiled chicken can be considered for the community as a food intake that can increase blood AMH so as to improve female fertility, ovarian function as well can slow down and delay the menopause process. This result is in line with research conducted by Kurniawan (2017) that AMH is a better marker for assessing women's reproductive age than chronological age[18]. AMH can be used clinically as a marker of ovarian aging and predict the final age of productive age appropriately compared to other markers, such as inhibin, estradiol and FSH dependent on the menstrual cycle.

CONCLUSION

It can be concluded that administration of *Gallus domesticus* boiled chicken white egg increased AMH level. The mean AMH level after treatment had a significant difference between control group (placebo) and treatment group with p = 0.001.

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