ORIGINAL ARTICLE:

Effect of ovarian autotransplantation on FSH levels in Wistar rats late menopause model

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ABSTRACT

Objectives: To determine the effect of ovarian autotransplantation on decreasing FSH level in Wistarrats late menopause model.
Materials and Methods: Experimental analytic research on 27 Wistar rats (Rattus novergicus) was divided into 3 groups. Group 1 (K1) or control. Group 2 (K2) performed bilateral oophorectomy without autotransplantation. Group 3 (K3) performed bilateral oophorectomy with autotransplantation. Measurements of FSH levels were performed on the first day, day 28 (four weeks after bilateral oophorectomy, during late menopause) and 56 day (four weeks after autotransplant). Measurement of FSH levels using ELISA. Data analysis used ANOVA and Post Hoc test.
Results: The mean FSH level measured on day 56 at K1 = 63.400 ng/mL, at K2 = 78.416 ng/mL and K3 = 31.991 ng/mL. There were significant differences between K1 and K2 (p = 0,000), K1 and K3 (p = 0,000), and between K2 and K3 (p = 0,000).
Conclusion: Ovarian autotransplantation decrease FSH levels in Wistar rats late menopause model.

Keywords: Autotransplantation; FSH levels; late menopause.

ABSTRAK

Tujuan: Menentukan pengaruh autotransplantasi terhadap penurunan kadar FSH pada tikus Wistar mode late menopause.
Bahan dan Metode: Penelitian eksperimental analitik pada 27 tikus Wistar (Rattus novergicus) dibagi menjadi 3 kelompok. Kelompok 1 (K1) atau kontrol. Kelompok 2 (K2) dilakukan bilateral ooforektomi tanpa autotransplantasi. Kelompok 3 (K3) dilakukan bilateral ooforektomi dengan autotransplantasi. Pengukuran kadar FSH dilakukan pada hari pertama, hari ke-28 (empat minggu setelah bilateral ooforektomi, pada masa late menopause) dan hari ke-56 (empat minggu setelah autotransplantasi). Pengukuran kadar FSH menggunakan ELISA. Analisis data menggunakan ANOVA dan Post Hoc test.
Hasil: Rerata kadar FSH yang diukur pada hari ke-56 pada K1 = 63,400 ng/mL, pada K2 = 78,416 ng/mL dan pada K3 = 31,991 ng/mL. Terdapat perbedaan signifikan antara K1 dan K2 (p= 0,000), K1 dan K3 (p= 0,000), dan antara K2 dan K3 (p=0,000).
Simpulan: Terdapat pengaruh autotransplantasi ovarium terhadap kadar FSH pada tikus Wistar model late menopause.

Kata kunci: Autotransplantasi; kadar FSH; late menopause.

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INTRODUCTION

Menopause is a natural and inevitable process that occurs spontaneously at the age of 40-58 years old in most of the women whereas an unnatural menopause is a condition marked by cessation of menstruation following bilateral oophorectomy, chemotherapy or radiation. The effect of such action may cause a woman to experience precog menopause that is menopause that occurred to women younger than 40 years old.[1]

The incidence of premature menopause also occurs in many cases of gynecological cancer undergoing chemotherapy and radiation.[2] The hypothalamic-pituitary-ovarian axis in which the production of steroid hormones by the ovaries will provide negative feedback on the hypothalamus-pituitary on the secretion of *Follicle-Stimulating Hormone* (FSH) and *Luteinizing Hormone* (LH) hormones. The gonadotropic cycle and steroid gonads will be replaced by monophasic cycles of increased gonadotropin secretion and decreased serum secretion and concentrations of estrogen, progesterone and inhibin hormones as a result of the absence of matured follicles at menopause. The cessation process of estrogen and progesterone secretion is particularly impactful systematically in the time of menopause.[3]

Follicle Stimulating Hormone is the first hormone to increase during menopause.[4]Loss of estrogen hormone in menopause causes several symptoms such as vasomotor disorder, genitourinary system disorder, sleep disturbance, psychological disorder, sexual disorder, and infertility. If it is chronic, it can cause osteoporosis and coronary heart disease. The emergence of meno-pausal symptoms lead to impaired life quality of a woman in running daily life,and one-third of women's live is passed through menopause.[5]The number of menopausal women worldwide is estimated to reach 1.1 billion people by 2025.[1]

Efforts that have been made at this time to treat menopause problem and to restore hormonal function among womenare by giving hormonal and non-hormonal therapies. Hormonal therapy by administering Hormone Replacement Therapy (HRT) is still the main recommendation for menopausal symptoms. However, HRT administration in menopausal women should be given carefullyand individualized considering the possible side effects.[1]

Young menopausal women who still want their reproductive function can receive donated oocytes in vitro fertilization (IVF) and ovarian donors. However, in Indonesia until recently oocyte donor or ovarian donor has not been accepted ethically and legally, so it cannot be done,andin some cases, the recipients show rejection reaction to the donated oocyte or the given ovary.[2,6] Efforts to maintain reproductive functionsin female can be done by freezing storing method of embryo, oocyte, or ovary. Ovary freezing have more advantages compard to oocyte and embryofreezing. This is because 90% of primordial follicles are present in ovarian cortex and are relatively resistant to cold trauma.[7]

This research will use female Wistar rats made into *late* menopause model by bilateral oophorectomy then measured FSH level after 4 weeks. The selection of female Wistar rats is because these animals have genetic and biological characteristic that resemble human. Female Wistar rats undergo spontaneous ovulation and shows regular and consecutive estrus cycle that may differ in age and species.[8] Rats breed rapidly and short-lived (2-3 years) so that several generations of rats can be observed in a short time. The use of experimental animals allows researchers to investigate the etiology and development of the disease in an impossible way against human, to perform procedures involving unethical damage to humans, and by using rats it is easier to handle, easier maintenance, at a cheaper cost.[9] The method of storing the frozen ovarian cortex selected in this research is vitrification considering its superiority for the effort of maintaining reproductive function that is more practical, fast, cheaper and causes minimal follicle damage because no ice crystals are formed.[10] This research aimed to determine the effect of autotransplantation on decreasing FSH level in Wistarrats late menopause model.

MATERIALS AND METHODS

This study was approved by the Institutional Review Board Gadjah Mada University. Yogyakarta, and the author have no conflict of interest. Analytical experimental study on the effect of ovarian autotransplantation on FSH levels by accessing differences in FSH hormone levels of late menopausal Wistar rats with atutotransplantation compared with the ones without autotransplantation. The research was conducted in June to August 2017 at the Integrated Research and Testing Laboratory of Gadjah Mada University (UGM) Yogyakarta. The sample of this research was a cortex of healthy ovarian tissue from Wistar female rats aged 10 - 12 weeks with weight of 200 - 250 grams. Treatment was given to 3 groups with the number of samples per group multiplied to 9, so that the total of the research sample was 27 rats. Independent variables used are bilateral oophorectomy and ovarian autotransplantation, while the dependent variable is FSH level.

The data analyzed using SPSS *software* (*Software Package for social Science*). Data analysis technique used in

this research is using *Levene* statistic test to assess the homogeneity of control group. Then followed by ANOVA (*Analysis of Varian*) test and followed by *post hoc test* with *Scheffe* test to find out the relation of FSH hormone levels before and after vitrification and after autotransplantation.

RESULTS AND DISCUSSION

This research uses data analysis technique by performing normality and homogeneity test data first then tested with ANOVA.

Table 1. Normality Test on FSH Data.

Research Variable	*P-value*	Information
FSH day 1	0,179	Normal Distribution
FSH day 28	0,078	Normal Distribution
FSH day 56	0,130	Normal Distribution

Table 2 above shows that p-value> 0.05 then FSH data on Day 1, Day 28 and Day 56. The three groups in this research are normally distributed.

Table 2. Data Homogeneity Test

Levene Statistic	df1	df2	Sig.
2,625	8	10,255	0,075

The result of homogeneity test using *Levene* test showed significance of p=0,075. Homogeinity test in this research has value of p>0,05 indicating sample of data obtained from homogenous population. Anova test were performed to test whether there were difference in mean of more than two test group. The result of the test in group 3 with autotransplantation showed that the significance value of p=0,001 with p<0,05 indicated that there is significant difference in the FSH level of group with autotransplantation. Therefore, it is necessary to do further Post Hoc test to know the relation of hormone levels of FSH before and after virtrification and after autotransplantation.

Based on the treatment group in the research, the observation result can be seen in Table 3. FSH levels of group 1 between day 1 and day 28 had a significance value of 0.910, group 1 between day 1 and day 56 had a significance value of 0.001 and group 1 between day 28 and day-56 has a value significance of 0.001.

Group 2 FSH levels between day 1 and day 28 had a significance value of 0.180, group 2 between day 1 and day 56 had a significance value of 0.001 and group 2 between day 28 and day 56 had significance value of 0.001. FSH levels of group 3 between day 1 and day 28 had a significance value of 0.001, group 3 between day 1 and day 56 had a significance value of 0.001 and group 3 between day 28 and day 56 has significance value of 0.001. There was significant decrease of FSH level in group 3 on day 28 and day 56 with p<0.05 that is FSH level decreased from 78,416 ng/mL to 31,991 ng/mL.

Table 3. Mean of Wistar Rats FSH Levels Based on Group 1, Group 2, and Group 3

Group	Day 1 (n=9) (ng/mL)	Day 28 (n=9) (ng/mL)	Day 56 (n=9) (ng/mL)	P Significance of p<0.05
Group 1 (Control)	9.073	12.675		0.910
	9.073		63.400	0.001*
		12.675	63.400	0.001*
Group 2 (Bilateral Oophorectomy)	13.823	19.499		0.180
	13.823		78.416	0.001*
		19.499	78.416	0.001*
Group 3 (Bilateral Oophorectomy and Autotransplantation)	2.611	43.472		0.001*
	2.611		31.991	0.001*
		43.472	31.991	0.001*

*significance of p<0.05

Table 4. Average FSH level of Wistar Rats based on Treatment in day 1, day 28 and day 56

Group	Control Group (ng/mL)	Bilateral Oophorectomy Group (ng/mL)	Bilateral Oophorectomy and Autotransplantation Group (ng/mL)	P significance p<0.05
Day -1 (n=9)	9.073	13.823		0.677
	9.073		2.611	0.235
		13.823	2.611	0.001*
Day -28 (n=9)	12.675	19.499		0.180
	12.675		43.472	0.001*
		19.499	43.472	0.001*
Day -56 (n=9)	63.400	78.416		0.001*
	63.400		31.991	0.001*
		78.416	31.991	0.001*

*significance p<0.05

Based on the research days, the results of the obser-vations can be seen in Table 4. The FSH level on day 1 between group 1 and group 2 did not have signifi-cant difference with value of p=0,677, group 1 and group did not have significant difference with value of p=0,235, and also group 2 and group 3 has significant difference with value of p=0,001.

The FSH levels on day 28 between group 1 and group 2 did not have significant differences with p = 0,180, group 1 and group 3 had significant differences with p = 0,001, and also group 2 and group 3 had significant differences with p = 0.001 .

FSH level of day 56 between group 1 and group 2 had significant difference with value of p=0,001, group 1 and group 3 has significant difference with value of p= 0,001, and also group 2 and group 3 has significant difference with value of p=0,001. There was significant decrease of FSH level in group 3 in day 28 and day 56 with p<0,05 that is FSH level decrease from 78,416 ng/mL to 31,991 ng/mL.

Blood sampling from experimental animals in all groups was conducted on day 1, group 1 was control group, bilateral oophorectomy was carried out in group 2 and group 3, while vitrification and freezing storing on ovary of experimental animal were carried out in group 3. Day 28 was calculated as four weeks after day 1 that blood sampling was taken from experimental animals throughout the group, and thawing and autotrans-plantation in ovaries of experimental animals of group 3were done. Day 56 was four weeks after day 28 of blood sampling from experimental animals in all groups.

Experimental animals in each group were three months old on the first day. Group 1 experimental animals on day 28 were still four months old. This is consistent with the previous studies that show the ovarian function of Wistar rats begins to decline at the age of 6 to 11 months.[11] Follicle Stimulating Hormone levels of group 2 and group 3 were above normal value. Increased FSH levels above normal values in group 2 and group 3 on days 28 were consistent to some previous research results indicating that surgical menopause with bilateral oophorectomy can significantly increase FSH levels.

Group 1 experimental animals on day 56 were five months old that is still within the reproductive age range of female Wistar rats. This is inconsistent with previous studies in which the ovarian function of Wistar rats begins to decline at the age of six to 11 months, and Wistar rats experience menopause at the age of 15-20 months.[11,12] Varying levels of FSH may be caused by the fact that researchers did not precisely calculate the experimental animal reproduction cycle at the beginning of the research where the mating process was ineffective because it was done only once at the beginning of the research, so the experimental animals in each group were not in the same reproductive cycle. Follicle Stimu-lating Hormone levels of female Wistar rats reached the peak in the proestruscycle and the lowest value in the diestrus cycle. The best way to determine this cycle is by vaginal swabs offemale Wistar rats and assessing cell differences according to their reproduc-tive cycle by using microscope.[13]

This research used ovarian cortex freezing storing method by means of vitrification method considering its superiority in maintaining reproductive function that is more practical, faster, cheaper, and causes minimal follicle damage because no ice crystals are formed.[10] Ovarian cortex freezing storingis considered easier to do than intact ovaryfreezingstoring. Vitrification method is an easy alternative to store freezing ovarian tissue.

Research results show no ultrastructure changes including core fragmentation, mitochondrial disorders, cell membrane shrinkage, and vacuole formation in cytoplasm of oocytes, follicular cells and stroma after undergoing vitrification and ovarian tissuethawing. Apoptotic changes are not found in ovarian cells at the ultrastructure level.[14]

Ovarian transplant studies in Indonesia are still limited to experiment animals because in terms of safety there is no clinical evidence that proves it is safe to do in humans, currently there is only a case report. The selection of using female rats was becausethis animal shows spontaneous ovulation that may occur and also shows a regular and consecutive estrus cycle that may be different from age and species.[15]

This research used female rats that were altered into a late menopause condition by applying bilateral oophorectomy. This research showed that freezing method with vitrification and continued by thawing and autotransplantationcould be performed on Wistar rats. This was indicated by significantly different FSH levels in groups with autotransplantationand without.

This research is a preliminary research, so there are still limitations in this research. Further research is needed to perfect our researchresults. Further research is expected to be synchronized to produce FSH levels from same cycle that can be done by vaginal smear examination.

CONCLUSION

There is an influence of autotransplantation to FSH levels in Wistarrats late menopause model. Autotransplantation decreases SH levels with significant difference $p=0.001$ ($p<0.05$) compared togroup without autotransplantation. This research is expected to be used as a further research to refine the results of this study and for the reference method of depreservation of reproduction in humans. In addition, it is needed to do injection of *Pregnant Mare Serum Gonadotropin* and mating for synchronization that aims to equalize the estrous cycle and vaginal smear examination at the time before the blood sampling of experimental animals.

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