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Follicular Analysis Graafian Follicle Due To Giving Red Fruit Oil Extract

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Abstract-*The occurrence of hormonal reproduction disorders in women is one of the risk factors for the occurrence of infertility influencing the process of folliculogenesis. Hormonal disorders need to be overcome through conventional medicine, one of which is Follicle Stimulating Hormone (FSH) hormonal therapy as a follicular development therapy for triggering ovulation. However, FSH therapy has some risks to be considered such as ovarian hyperstimulation side effects, high maintenance costs, emotional burdens, and unsatisfactory results. Thus, it requires other solutions which are more economical and have no side effects. Red fruit or Pandanus Conoideus is a typical Papuan plant that has many compounds. One of which has essential compounds needed for the female reproductive system. This study analyzes the amount, diameter, and area of Graafian Follicles after being given red fruit oil extract in infertile female mice. The results showed that the red fruit oil extract had an effect on the number, diamater, and area of Graafian Follicles with p value <value α (0,05) and the value of the correlation coefficient (r) between the diameter and area of the follicle is 0,88 indicating that there is a close relationship between the diameter variables and the width of the de graaf follicle.*

Keywords*: **Folliculogenesis process; Graafian Follicle; Red fruit***

I. Introduction

Being a mother is a dream for every married woman because only women can give births. However, there are issues regarding women's inability to do so. The inability of a woman or married couple to have a child can be caused due to the occurrence of infertility. But the inability of a woman or married couple to have a child can be caused due to the occurrence of infertility[1]. Infertility is a failure to to conceive a child in married couples within a certain period of time due to some impaired reproductive function[2,3].

The incidence of infertility in both women and men is estimated between 8 – 12% of all reproductive age couples in the world[4]. The incidence of infertility for women of reproductive age in Asia reaches 30%[5] and for men is 20-30% of cases of infertility[6]. Based on the data from the Indonesian Central Bureau of Statistics the (BPS) the prevalence of infertile events in 2013 from all existing partners was 15-25%[7].

The reason of infertility caused by female factors is 65%, male factor is 20%, and other unknown factors is 15%[8]. The risk factors that cause female infertility are caused by the characteristics factors, nutritional status, lifestyle, psychological, infections, reproductive organs disorders, and hormonal disorders[9,10]. The occurrence of hormonal disorders of reproduction is one of the risk factors for the occurrence of infertility due to reducing or obstructing hormones secreted[10].

The hormonal disorders cause disruption in the process of folliculogenesis due to Gonadotropin Releasing Hormone (GnRH) which suppresses the secretion of FSH[11]. FSH hormone is a stimulating hormone for follicular maturity and ovulation[11]. Decreased FSH hormone secretion stimulates the death of granulosa cells in ovarian follicles and the disruption of folliculogenesis processes and results in a decrease in follicular production in graaf [13,14].

The decreasing in the amount of de graaf follicle production is caused by the disruption of the folliculogenesis process which gives the effect of not occurring ovulation or it releases one or more oocytes in the reproductive cycle so that it affects the occurrence of infertility[15]. Infertility occurs due to hormonal imbalance which results in impaired FSH thus, it needs to be overcome through conventional treatment with reproductive technology through FSH hormonal therapy as a follicular development therapy for triggering the ovulation[12,16]. However, the conventional treatment of FSH therapy in women who experience hormonal disorders has some consequences such as in therapy, high maintenance costs, emotional burden, and unsatisfactory therefore, other solutions which are more economical and have no side effects are needed[17].

It is estimated that around 80% of people use traditional herbs as fertility drugs for both women and men[18]. Red fruit or *Pandanus Conoideus* is a typical Papuan plant that has been consumed by Papuans every day which has many compounds that are beneficial for Commonly, red fruit is processed into oil and pasta[19], besides it can also be used as an alternative to overcome fertility problems in women because it contains betacarotene reaches 700 ppm and the content of tocopherol reaches 11,000 ppm[20]which is an essential compounds needed for the reproductive system of women[21].

Based on the results of research on infertile mice given red fruit oil extract, the results showed. There are

previous studies analyzed the infertility on mice which was treated by giving red fruit oil extract. The results showed that administration of red fruit oil extract can improve the process of folliculogenesis by increasing the number of graafian follicles[22]. The high content of beta-carotene and tocopherol in red fruit as essential compounds can stimulate more secretion of FSH hormone so that it helps in the process of follicular development[23,24].

The process of folliculogenesis is characterized by increasing follicular As a result, it produces mature oocytes and ovulation occurs due to the influence of Luteinizing hormone (LH). Graafian follicles is the final stage of follicular development from the process of folliculogenesis that is easily observed and distinguished from other follicles because of its large size or diameter and its large antrum[11].

The measurement of the asymmetrical graafian follicles diameter is determined by calculating the average of two or more of the widest cross-sectional measurements[25]. The asymmetrical form of graafian follicles influences the size of the follicular. Thus, it is necessary to measure the width of the graafian follicles to obtain a value for the surface size of the graafian follicles.

Currently, the using technology development enables the measurement of the de graaf follicle area utilizing a technique known as digital image processing techniques. Digital image processing is image processing or objects using computerization utilized to produce a higher quality output image since it has high and efficient computing capabilities as a result, the information can be interpreted better[26]. One of the benefits of image processing is identifying the area of an object. Extensive calculation of an image or object has some benefits, one of which is in calculating or observing the area or number of cell morphology and or bacteria[27,28,29].

The measurements using digital image processing techniques can provide better accuracy in number and size[26]. Measuring the area of graafian follicles using digital image processing techniques is taken as an effort to prove the usefulness of the effect of red fruit oil extract in stimulating the development of follicles into de follicles so that ovulation can occur which can affect the occurrence of pregnancy.

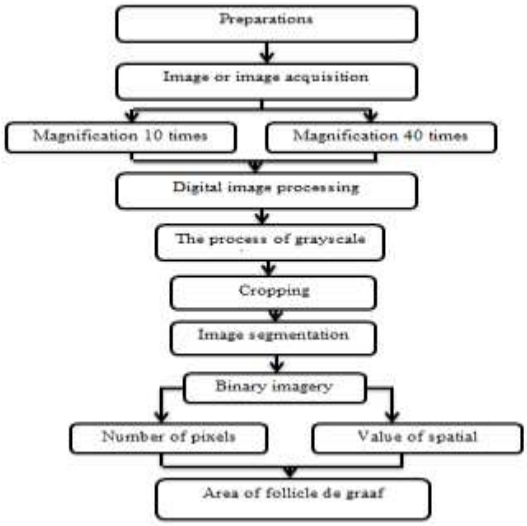
II. Method

This research is was a true experimental research with a Post Test given limitedly to the control group. It was conducted at the Animal Biology Laboratory of Semarang State University in Indonesia. This study used 4, two groups were control group without being given red fruit oil extract named as negative control group (K1) and positive control group (K2). the negative control group consisted of female mice and while the positive control group consisted of female mice used as the infertile models. There were two other intervention groups consisting of female infertile mice as the models which were given the red fruit oil extract with different doses of 0,05 mL (E1) and 0,1 mL (E2).

The population was female mice *(Mus Musculus)* with 24 mice as the sample that met the inclusion criteria based on randomization. They were divided into 4 groups consisting of 6 female mice in each group. The intervention group were given red fruit oil extract in different doses which is 0.05 mL and 0.1 mL with the administration time 1 time per day for 15 days. However before being given the red fruit oil the positive control group and the intervention were used as an infertile model by giving an injection of a combination of testosterone hormone in a dose of 1 mg / kg BB as much as 0,02 mL subcutan every day for 14 days.

After the administration of red fruit oil extract for 15 days in the intervention group, the second ovary organ samples were taken using HE staining to analyze the post test The post test results covered the details of the number, diameter, and area of graafian follicles both intervention and control groups.

The number of graafian follicles were analyzed on both ovaries of mice using a digital microscope with a 10 and 40 times and the diameter of the graafian follicles was analyzed using computer software connected with digital microscopes which had calibration values based on magnification of the object used. The diameter of the graafian follicles was measured starting from the external or outer layers of theca cells by calculating the average diameter obtained from the longest diameter and the shortest diameter of the graafian follicles, and the area of graafian follicles were analyzed using the digital image processing technique. The area of follicles was analyzed by processing the image of graafian follicles with a 40 and 10 times magnification. Follicle images are labeled to differentiate follicle images in the control groups and in the intervention groups. It also differentiated the magnification used. After that, the follicle images were processed.



In the pre-processing stage, the colored follicular images were converted to a gray scale, the image on a gray scale will be cropped or cut to determine the boundaries of the graafian follicles observed. In the next stage, the image was segmented to distinguish between objects and their background. In this stage, thresholding was done with the binary image in which the value of white object was 1 while the black object was 0. Then, the follicle area was

analyzed by considering the number of pixels in the image multiplied by the value of the spatial resolution of the image. The value of spatial resolution used was from the example of the Phantom object measuring the value of 1 pixel resolution based on the magnification object used. The image of de graaf follicles with a 10 times magnification had a spatial resolution value of 1,2 µm/pixel while the 40-times magnification of the scale value used was 0,93 µm/pixel. The hypothesis proves that red fruit oil extract can affect the number of graafian follicles analyzed using the Kruskal Wallis test and prove that red fruit oil extract can affect the diameter and area of graafian follicles analyzed using ANOVA test. It was proving that the correlation between diameter and the area of de graaf follicles analyzed using Bivariate Pearson correlation test.

III. Results & Analysis

The results of the univariate data analysis described the mean and standard deviation of the research variables, while the bivariate test analysis was carried out to analyze the effect of giving red fruit oil extract to the number, diameter, and area of the follicle using the ANOVA. The analysis was followed by the Post Hoct test to find significant differences between groups and also analyzed the correlation test of the diameter and the area of the graafian follicles using the Bivariate Pearson correlation test. The bivariate and correlation test were carried out when the data were normally distributed and homogeneous with a value (p value> 0,05).

The analysis results of the effect of red fruit oil extract (Pandanus Conoideus) and the significant differences between groups on the number of graafian follicles in female mice are presented in Table 1.

Table 1. The effect of fruit oil extract *(Pandanus Conoideus)* and the significant differences between groups on the number of the graafian follicles in female mice

No	Group	The number of the graafian follicles (mean±SD)	*P value*	*P value (PH Tests)*
1	K1	2,00 ±0,00	0,006	E2 to K2 0,000
2	K2	1,8 ±0,44		
3	E1	3,4 ±1,81		
4	E2	6,6 ±2,19		

Information : *P value and Post Hoc Tests: significance<0,05

Based on Table 1, it shows that there is an effect of giving red fruit oil extract to the number of the graafian follicles this is evidenced by the results of the p value through the Kruskal Wallis test which is 0,006 (p <0,05). In addition, there are significant differences in the number of de follicles between the intervention groups (E2) given red fruit oil extract with a 0.1 mL dose and with positive control group (K2). It is shown from the results of the Post Hoc Test with a p value of 0,000 (p <0,05).

The analysis results of the effect of red fruit oil extract *(Pandanus Conoideus)* and the significant differences between groups on the graafian follicles diameter in female mice are presented in Table 2.

Table 2. The effect of fruit oil extract *(Pandanus Conoideus)* and the significant differences between groups on the graafian follicles diameter in female mice

No	Group	The diameter of the graafian follicles (µm)(mean±SD)	*P value*	*P value (PH Tests)*
1	K1	223,26±57,88	0,01	E1 to K2 0,016
2	K2	170,84±39,02		
3	E1	268,6±35,1		
4	E2	201,9±38,4		

Information : *P value and Post Hoc Tests: significance<0.05

Based on Table 2, it can be seen that there is an effect of giving red fruit oil extract to the graafian follicles diameter, it is evidenced by the results of the p value through anova test at 0,01 (p <0,05). In addition, there are significant differences in the graafian follicles diameter between intervention groups (E1) given red fruit oil extract with the dose of 0,05 mL and with the positive control group (K2). This is evidenced by the results of the Post Hoc Test with a p value of 0,016 (p <0,05).

The analysis results of the effect of red fruit oil extract (Pandanus Conoideus) and the significant differences between groups on the area of the graafian follicles in female mice are presented in Table 3.

Table 3. The effect of fruit oil extract *(Pandanus Conoideus)* and the significant differences between groups on the width of graafian follicles in female mice

No	Group	The Average of the graafian follicular area (mm^2) (mean±SD)	*P value*	*P value (PH Tests)*
1	K1	0,042±0,022	0,006	E1 to K2 0,006
2	K2	0,024±0,010		
3	E1	0,069±0,022		
4	E2	0,033±0,011		

Information : *P value and Post Hoc Tests: significance<0,05

Based on Table 3, it can be observed that there is an effect of giving red fruit oil extract to the graafian follicles area, it is proven by by the results of the p value through the ANOVA test of 0,006 (p <0,05). In addition, there were significant differences in the width of the graafian follicles. It is proven by between the intervention groups (E1) given the red fruit oil extract with the dose of 0,05 mL and with the positive control group (K2). This was shown by the results of the Post Hoc Test with a p value of 0,006 (p <0,05).

During the measurement, the graafian follicles is still measured by using a digital microscope in the form of the graafian follicles which is not symmetrical . Therefore, the measurement of de graaf follicle area utilizing digital

image processing techniques to determine the correlation of the graafian follicles diameter size which is not symmetrical with the graafian follicles size measured using digital image processing techniques.

The analysis results of the correlation of the graafian follicles diameter to the area of the graafian follicles in female mice using digital image processing are presented in Table 4.

Table 4. The correlation of the diameter and the graafian follicles area in female mice using digital image processing

No	Variable Correlation	*P value*	*Pearson Correlations (r)*
1	The Diameter of follicle de graaf (X)	0,000	0,880
2	Area of follicle de graaf (Y)		

Based on Table 4, the results of the study shows that there is a significant correlation between the diameter and the de graaf follicle area. It is proven by the results of the p value through the Pearson Bivariate Correlation test of 0,000 (p <0,05). In addition, based on the value of r, the relationship of diameter with the area of the graafian follicles is 0,88> r which In the table, the value shown is 0.444 meaning that there is a correlation between variables with broad variables. The r or Pearson Correlation value in this analysis is positive meaning that the relationship between the two variables is positive.

The results showed that there was an effect of giving red fruit oil extract to the number, diameter, and area of the graafian follicles (p = <0,05). From the results of the hypothesis, the research shows that the administration of red fruit oil extract with different doses can significantly affect the number, diameter, and area of the graafian follicles.

One of the consequences of hormonal disorders is that it causes disruption of the folliculogenesis process, which affects the maturity of follicles It may affect the occurrence of anovulation as well[27]. Therapy that can be given for the case is GnRH therapy containing FSH to influence the maturity of oocytes to stimulate ovulation[28]. The red fruit oil extract is an alternative as FSH hormone therapy which is more economical and has no side effects that can stimulate the development of follicles into the graafian follicles stimulating ovulation [11].

The healthy the graafian follicles will be larger in size and can be ifferentiated along with its development to reach the preovulatory stage. The size of the de graaf follicle determines the development of oocytes or ovum and affects ovulation. The healthy de graaf follicles are characterized from its large size that will affect the proliferation of granulosa cells and theca cells[29]. The results of the study show that granulosa cells play an important role in the development of oocytes because they function in providing nutrients, metabolism, and gene expression in oocytes and blocking the polyspermia[29].

However, it is often found that the diameter of graafian follicles is not symmetrical so that the measurement of the asymmetrical de graaf follicle diameter is determined by calculating the average of the two widest cross-sectional measurements[25]. The results of this study indicate that the diameter of de graaf follicle has a symmetrical shape characteristic. During this time, the measurement method used to measure the diameter of the follicle which is not symmetrical by using a digital microscope. In this study, a new method was carried out, namely measuring follicle area using digital image processing methods. The measurements of the follicular area are carried out to provide a measurement of the broad value of the follicle because it has an asymmetrical shape.

Based on the results of measurements of the follicle de graaf area using digital image processing techniques, it showed that the administration of red fruit oil extract with a dose of 0,05 mL can affect the area of the graafian follicles. In addition, there is a positive correlation between the diameter and area of the follicle de graaf with a value of r 0,88 which indicates that there is a close relationship between variable diameters and the area of follicles de Graaf[30]. The normal size of de graaf follicular diameter in mice is starts from 329 µm[25]. Based on some previous research, the follicles with a normal diameter size of 329,9 µm having a broad size of de graaf follicles measured using digital image processing, the follicular area value was 0,080 mm^2 so that the size of a de graaf follicle with a size of 0,080 mm^2 could be a predictor in influencing oocyte or ovum maturity and affect ovulation.

Red fruit oil extract contains nutrients, namely energy, fat, fiber, calcium, phosphorus, iron, vitamin B1 and vitamin C, nialine and water which can affect follicular development. The Follicles will develop and continue to grow when they are supplied with nutrients or important food obtained through blood from vitamins and steroid hormones. However, there are some elements that cannot be supplied by most enzymes, antigens, antibodies and protein hormones[31].

Besides, the content of beta-carotene and tocopherol in red fruit oil extract are essential compounds needed for the female reproductive system[21]. Betacarotene and tocopherol contain high antioxidants in red fruit which are useful in preventing various diseases[32]. Betacarotene and tocopherol are the types of vitamins that are absorbed in the small intestine and enter the body's circulation through the lymphatic system, absorbed with lipids in the form of kilomicron and head to the liver. After that, betakroten and tocopherol will be penetrated together with blood circulation to tissues that have a surface of saturated fat, namely in the brain[32].

Betacarotene and tocopherol in the brain stimulate the hypothalamus and give stimulation to the pituitary or pituitary gland. The brain will signal the pituitary gland through GnRH neurons to secrete more FSH[11]. The high level of FSH will affect the growth and development of follicles, preventing disruption in the amount of maturity of the follicles, helping the oocytes become more mature which affects the occurrence of fertilization or pregnancy[23]. Granulosa beta-carotene cells are converted into retinol, which affects the quality of follicles, retinol my support can help the growth and development of

follicles which can affect the maturity of oocytes and follicular metabolism which affect fertilization[25].

IV. Conclusion & Recommendation

As previously discussed, the results have been described. To sum up, the study finds that red fruit oil extract with a dose of 0,1 mL can affect the number of de graaf follicle. On the other hand, a dose of 0.05 mL can affect the diameter and the area of the graafian follicles. The normal diameter of the graafian follicles can be used as a parameter in measuring the graafian follicles where the size of the graafian follicles is 0,080 mm^2 measured using digital image processing server as a predictor factor in influencing the maturity of oocytes or ova, which affects ovulation. The digital image processing technique is a new method utilizing technology in the health field that can be used to measure the extent of a follicle. In the future, it is expected that technological opportunities can be more widely used in the health field so that it can make a more efficient and accurate contribution to health services.

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