

Endometrial Angiogenesis On Guinea Pig (*Musmusculus*) With Cigarette Smoke Exposure

Sugijati, I G.A. Karnasih, JeniePalupi
Health Polytechnic of Malang, Republic of Indonesia

Abstract: *This research generally aims to study the effect of cigarette smoke on guinea pig with endometrial angiogenesis. This research is a laboratory experiment with posttest design using experimental and control groups (post-test only control group design). The study was conducted to subjects with a variety of ferns smoke K Group as a control group that was not given smoke. Treatment group (P) was divided into P1 as the group given cigarette smoke as much as 1 pack/24 hours. P2 group was given 2 packs of cigarette smoke/24 hours. P3 group was given 3 packs of cigarette smoke/24 hours. Treatment in group P1 to P3 is given once in a day. Surgery was done after day 12 to take its endometrium. Then, it was examined to observe its morphology. The result of those 4 treatment groups were compared with control group. Based on the calculation, it was obtained that the sample size of 40 guinea pigs were divided into 4 groups, each group consisted of 10 guinea pigs. To divide the sampling, the researchers used complete random sampling technique. The results showed that exposure of cigarette smoke affect significantly against endometrial angiogenesis on guinea pigs (*Musmusculus*) with F value of 3.186 and a significant value of 0.034. Exposure to higher amount of cigarette smoke will further decrease the thickness of the endometrial angiogenesis.*

Keywords: *Angiogenesis endometrium, Exposure to cigarettes smoke*

I. Introduction

Reproduction health is a hope for every human being, especially for a woman. Reproduction is synonymous with the image of a woman, because if a couple does not have offsprings, then the society will put the blame on the women. A woman's reproductive health is determined by the condition of the female reproductive organs. Endometrium as one of the reproductive organs holds an important role in in. Adult women experiencing problems relating to the condition of the endometrium, such as menstrual disorders, endometrial polyps, endometriosis, abortion and cancer. Endometrium can function well if angiogenesis takes place in accordance with its reproductive cycle.

Endometrial Angiogenesis is a process of blood vessels formation in the endometrium. The process of new blood vessel formation is influenced by pectan vascular endothelial growth factor (VEGF) (Ferrara and Davis-Smyth, 1997). Endometrial disorders or angiogenesis disorders can affect endometrial function or can cause endometrial infertility. Its function is influenced by the secretion of estrogen and progesterone hormones. Eugene's (2002) study found out that estrogen increases the incidence of angiogenesis endometrium. Infertility problems prevalence in the world each year is 1 of 7 couples. Infertile couples in Indonesia in 2009 was 50 million couples or about 15-20% from all couples (Inasoengkowo, 2009).

Kelly (2006) also found out that angiogenesis endometrial disorders can be caused by lifestyle such as age, history of ectopic pregnancy, obesity, and smoking. Smoking today is a very familiar sight. Smoking habits give pleasure to the smoker, but on the other hand it can cause negative effects for the smokers themselves and the people around them. The impact will be worse for the smoker himself. Cigarettes with toxic substances contain one of the factors that influence the secretion of estrogen. Direct toxic effects of tobacco smoke are still unclear. Therefore the researchers wanted to examine what are the effects of cigarette smoke exposure on women's angiogenesis. Some researchers suspect that the smoke has the effect of lowering the risk of endometrial cancer. This is presumably due to the smoke which allegedly reduces the production of estrogen and also the antiestrogenic effects of smoking. Another theory says that smoking affects the absorption, metabolism and distribution of estrogen. Smoking is also thought to change more estrogen into 2-hidroksiestrone with low estrogenic effect.

General Objective: This research generally aims to study the effect of cigarette smoke exposure on endometrial angiogenesis on guinea pigs (*Musmusculus*).

Specific Objectives

1. To study the endometrial angiogenesis on guinea pigs (*Musmusculus*).
2. To study the endometrial angiogenesis on guinea pigs (*Musmusculus*) with exposure to cigarette smoke.
3. To study the effect of cigarette smoke exposure on endometrial angiogenesis on guinea pigs (*Musmusculus*).

Benefits

The results of this study are expected to contribute to the development of prevention of health problems, especially for disorders of the female reproductive endometrium. In academic areas, it can provide a broader and in-depth understanding of endometrial disorders.

Method

Design

This research is a laboratory experiment with post-test design with experimental and control groups (post test only control group design) (Zainuddin, 2000). The schematic design of this study can be described as follows:

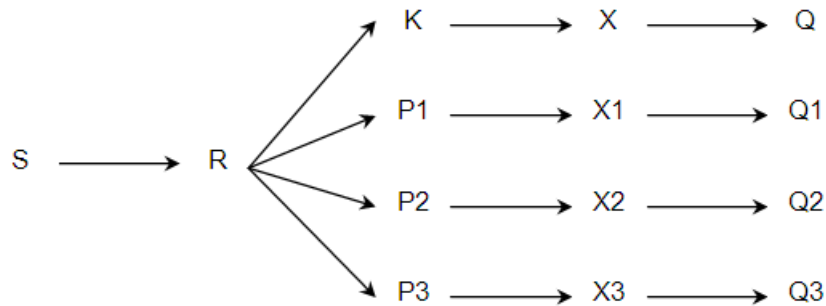


Figure 1 Chart of Research Plan Specifications:

Samples: Randomization

- K: The control group was given aroma therapy smoke
- P1: The treatment group I were given smoke from 1 pack of cigarette/24 hours
- P2: The treatment group I were given smoke from 2 pack of cigarette/24 hours
- P3: The treatment group I were given smoke from 3 pack of cigarette/24 hours
- X: Control
- X1: Treatment I
- X2: Treatment II
- X3: Treatment III
- Q: The results of the control group
- Q1: Results of Treatment I
- Q2: Results of treatment II
- Q3: Results of treatment III

The study was conducted in subjects with a variety of ferns smoke K Group as a control group given the aroma therapy smoke. Group P1 was given smoke from 1 pack of cigarette/24 hours. Group P2 was given smoke from 2 pack of cigarette/24 hours. Group P3 was given smoke from 3 pack of cigarette/24 hours. The treatment in group P1 to P3 was given once a day. After 12 days of treatment for, a surgery was conducted to take its endometrium. The result was used to observe its morphology. The thickness of those four examination of the treatment group were compared with the control group.

Research sample

28 guinea pigs were divided into 4 groups, each group consisting of 7 guinea pigs. A complete random sampling technique was used.

Hypothesis

There was no difference on endometrial angiogenesis on guinea pigs (*Mus musculus*) with exposure to cigarette smoke.

II. Results & Discussion

Results

a. Univariate analysis

Descriptive analysis for endometrial angiogenesis thickness on guinea pigs by exposure to cigarette smoke on each treatment are presented in the following table:

Table 1 The average value and standard deviation on endometrial angiogenesis on guinea pigs by exposure to cigarette smoke

Cigarette smoke exposure	n	Average	Standard Deviation
Control	11	196.188	67.514
1 pack/24 hrs	11	176.136	69.093
2 packs/24 hrs	11	168.046	61.299
3 packs/24 hrs	11	119.591	38.648
Average	11	164.989	64.821

Source: Reports

Based on Table 1, the results showed that the highest thickness of the endometrial angiogenesis was produced by the control group, followed by treatment groups with exposure to cigarette smoke 1 pack/24 hours, 2 packs/24-hour and 3-packs/24 hours. This shows that with increasing exposure to cigarette smoke will result in a decrease in the thickness of the endometrial angiogenesis. Giving exposure to cigarette smoke resulted in a decrease in the thickness of the endometrial angiogenesis, that is the exposure to cigarette smoke 3 packs/24 hours. The average thickness of the endometrial angiogenesis in cigarette smoke exposure is presented in Figure 4.1.

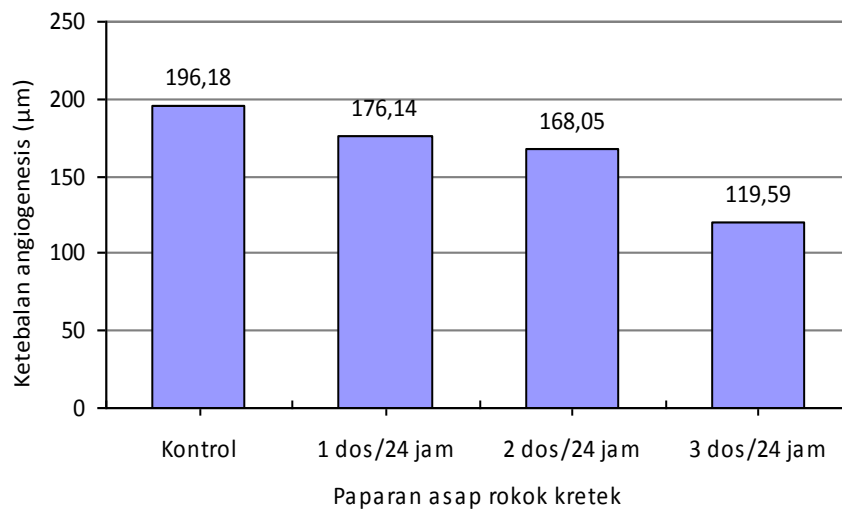


Figure 2 Histogram of average thickness of the endometrial angiogenesis with various cigarette smoke exposure

Bivariate analysis

The result of mean difference score of endometrial Angiogenesis thickness

Mean difference test is used to determine whether the treatment group with cigarette smoke exposure have different effects on angiogenesis endometrial thickness of guinea pigs. Based on data normality test, this study used one way analysis of variance (Oneway ANOVA) with one factor. The result of multivariate analysis on cigarette smoke exposure treatment for endometrial angiogenesis thickness are presented in Table 2

Table 2 Results of multivariate analysis of angiogenesis endometrial thickness on guinea pigs with exposure to cigarette smoke

Source of variations	Square value	Degrees of freedom	Mean square	F-calculation	Sign.	Note
Treatment	34843.426	3	11614.475	3.186	0.034	Significant
Control	145831.3	40	3645.783			
Total	18674.7	43				

Source : attachment

Based on the above table, the results obtained from multivariate analysis was F-calculated value of 3.186 with a significance value of 0.034. Significance value of 0.034 is smaller than α (0.05). It shows that the treatment of cigarette smoke exposure significantly influence the thickness of the endometrial angiogenesis.

The test was followed by Tukey-HSD test, because the results of analysis of variance demonstrated a significant effect. The results of Tukey HSD test about the influence of cigarette smoke exposure on angiogenesis endometrial thickness are presented in Table 4.5.

Table 3 HSD test results of endometrial angiogenesis thicknesson guinea pigs treated with cigarette smoke exposure

Cigarette smoke exposure	Endometrial angiogenesis thickness (µm)
Control	196.182 a
1 pack/24 hours	176.136 ab
2 packs/24 hours	168.046 ab
3 packs/24 hours	119.591 b

Source :attachment

HSD-Tukey test results on endometrial angiogenesis thickness which were affected by cigarette smoke exposure (Table 3) indicating that the control group had no significant difference with cigarette smoke exposure treatment with 1 pack/24 hours and 2 packs/24 hours, but significantly different from treatment exposure to cigarette smoke 2 packs/24 hours. Treatment of exposure to cigarette smoke of 1 pack/24 hours and 2 packs/24 hours did not differ significantly with treatment exposure to cigarette smoke of 2 packs/24 hours. Treatment of exposure to cigarette smoke with 3 packs/24 hours resulted in the thickness of the thinnest endometrial angiogenesis with the amount of 119.591 µm.

III. Discussion

Oxidants in cigarette have sufficient quantities to play a major role in the occurrence of damage to the airways. It is known that tobacco smoke oxidants consume intracellular antioxidants in lung cells (in vivo) through a mechanism linked to oxidant stress. It is estimated that each puff of a cigarette has oxidant ingredients in a very large number, including aldehydes, epoxida, peroxide, and other free radicals which may live for quite long and able to survive to cause damage to the alveoli.

The results showed that the highest thickness of the endometrial angiogenesis was produced by the control group (196.18 µm), followed by the treatment group with exposure to cigarette smoke of 1 pack/24 h (176.14 µm), 2 packs/24 h (168.05 µm) and 3 packs/24 h (119.59 µm) in successive order. This shows that increasing exposure to cigarette smoke will result in a decrease in the thickness of the endometrial angiogenesis.

This means that cigarette smoke consists of 4000 chemical substances and 200 of them are poisonous, among others are carbonmonoxide (CO) which is produced by cigarette smoke and can cause vessels to become rigid, so that the blood pressure rises and the blood vessel walls can be torn. CO can also cause hemoglobindesaturation, directly lowering oxygen circulation for the entire body including endometrialangiogenesis. CO replaces oxygen in hemoglobin, interfering with the release of oxygen and quicken atherosclerosis (calcification or blood vessel walls thickening). Nicotinalso stimulates blood pressure increase. Nicotin inactivates trombocytes which cause trombocyte clotting to blood vessel walls. Nicotin, CO other substances in cigarette smoke has proven to be able to ruin endothelial vessel (the wall inside blood vessel), causing blood clotting easier to happen so that it can damage blood vessels which causes imperfect endometrial angiogenesis.

Analysis of variance test resulted in F-calculation value of 3.186 with a significance value of 0.034. Significance value of 0.034 is smaller than α (0.05). This shows that the treatment of cigarette smoke exposure significantly influence on the thickness of the endometrial angiogenesis. Estrogen is a female reproductive hormones in animals. This hormone mainly secreted by the follicle cells and initiate constituent granula on endometrial growth. Reproductive cycle in guinea pigs consists of four phases, namely proestrus, estrus, and diestrus. Proestrus is marked by oval, blue with red cell nucleus on vaginal smear of the epithelial. Estrus phase is characterized by epithelial cells which undergo thickening on its epithelial cells. Metestrus phase is then connected with diestrus phase which shows nucleated epithelial and vaginal mucus.

Estrogen is formed by the granulosa cells in ovarian follicles through a series of conversions through enzymatic process. The main substance forming estrogen is cholesterol that undergo changes to be estrogen.

Sirajudin (2011) says that cigarette smoke is a pollutant that strongly influences the production of estrogen. Cigarette smoke contain free radical compounds in a large number namely CO, Tar, Nicotine that affect the nerve cells in the brain. Cells affect the secretion of estrogen to decrease.

Other studies have shown significant differences in the control and treatment groups due to the treatment group receiving exposure to cigarette smoke containing free radicals that trigger oxidative stress, causing damage to mucociliary clearance. Vibrating hairs, cough reflex and alveolar macrophages can not function properly to dispose particles or bacteria which gets into the lungs thereby increasing the risk of infection and inflammation in the lungs. According to research conducted by Bannerjee, et al (2004), cigarette smoke causes oxidative damage to the lung alveoli. Oxidants contained in cigarette smoke cause significant oxidative damage to protein microsomes and increase the occurrence of proteolysis caused by an imbalance between proteases and anti-proteases. Proteolysis and oxidative damage will cause damage to the alveolar wall and will eventually ruin the whole lung.

Uterine in which there is endometrium which functions as a reproductive organ that has estrogen receptors so that changes in the constituent layers of the endometrium regulates hormone, in this case estradiol. The result of this study showed that if guinea pigs are more exposed to cigarette smoke then they are increasingly stressed so that endometrium becomes thinner as estrogen initiated endometrial growth.

The worst effect for human beings when exposed to cigarette smoke is it can cause the endometrium not to grow as it should be so that the threat of infertility will occur.

The limitation of this study was that pap smear to guinea pigs was not done prior to the study, so that at what cycle the guinea pigs were unknown since each cycle probably has different thicknesses.

IV. Conclusion And Recommendation

Conclusion

Based on the results of research and discussion about "Endometrial Angiogenesis of guinea pigs (Musmusculus) with exposure to cigarette smoke", it can be concluded as follows:

1. The average thickness of endometrial angiogenesis on guinea pigs (Musmusculus) without cigarette smoke exposure treatment (control) reaches the number of 196.188 ± 67.514 μ m.
2. The average thickness of endometrial angiogenesis on guinea pigs (Musmusculus) with treatment of cigarette smoke exposure was equal to 176.136 ± 69.093 μ m (1 pack /24 h), 168.046 ± 61.299 μ m (2 packs/24 hours) and 119.591 ± 38.648 μ m (3 packs/24 hours).
3. Exposure to cigarette smoke affects significantly against endometrial angiogenesis on guinea pigs (Musmusculus) with the value of the F-calculation equal to 3.186 and a significance of 0.034. Giving exposure to smoke cigarettes with higher amounts will further decrease the thickness of the endometrial angiogenesis.

Recommendation

Based on the results, discussion and conclusions above, it is recommended that:

1. There needs to be more research on the effects of cigarette smoke toward fetal development.
2. There needs to be more research on the effects of cigarette smoke toward angiogenesis in pituitary.
3. Epidemiological studies should be carried out in human about the effect of cigarette smoke toward infertility in men.

References

- [1]. Campbell DT dan Stanley JC, 1963. *Experimental Design for Research Rand*. Chicago : Mc Nally College Publishing Company.
- [2]. Cochran W.G, 1991. *Teknik Penarikan Sampel, Edisi Ketiga*. Terjemahan Rusdiansyah. Jakarta : Penerbit Universitas Indonesia.
- [3]. Dorland, 2005, *Kamus Kedokteran, Edisi kedua puluh delapan*, EGC, Jakarta. Hal 726 - 727.
- [4]. Ganong WF, 1999. *Review of Medical Physiology*, 19th ed. Stand Ford Connecticut:
- [5]. Ganong, WF. 2003, *Buku Ajar Fisiologi Kedokteran, Edisi 20*, EGC, Jakarta Hal.417-431.
- [6]. Greenspan FS and Forsham PH, 1997. *Basic and Clinical Endocrinology*, 5th ed. Stand Ford Connecticut :Appleton and Large, pp 545-567.
- [7]. Guyton dan Hall, 1997, *Buku Ajar Fisiologi Kedokteran Cetakan 1*, EGC, Jakarta, Hal; 1283-1302.
- [8]. Hardjo Pronjoto, 1995. *Ilmu Kemajiran Ternak*. Surabaya: Airlangga University Press.
- [9]. Heyne K, 1987. *Tumbuhan Berguna Indonesia*, Jilid 11. Jakarta :Badan Penelitiandan Pengembangan Kehutanan RI, hlm 840-842.
- [10]. Hunter RHF, 1993. *Fisiologi dan Teknologi Reproduksi Hewan Betina Domestik*. Bandung: Penerbit ITB.
- [11]. Ismudiono, 1999. *Embriologi Reproduksi pada Ternak*. Surabaya: FKH Universitas Airlangga.
- [12]. Johnson M, 1995. *Essential Reproduction*, 4th Edition. London: Department of Embriology and Department of Experimental of Psychology University of Cambridge.
- [13]. Limbong T, 2004, *Pengawas Ekstrak Etanol Kulit Batang Pakettu (Ficus super bamiq) Terhadap Foklulogenesis Ovarium Mencit (Musmusculus) Tesis*, Universitas Airlangga Surabaya.
- [14]. Marge K, Judith T and Gay, 1997. *Kesehatan Wanita*. Yogyakarta: Universitas Gajah Mada.
- [15]. Mursito B, 2001. *Ramuan Tradisional untuk Kesehatan Ibu Hamil*. Jakarta Penerbit Swadaya.
- [16]. Partodihardjo S, 1992. *Ilmu Reproduksi Hewan*. Jakarta: Penerbit Mutiara, hlm 43-52; 105-108; 173-181.
- [17]. Prawirohardjo, 1991. *Ilmu Kebidanan, Cetakan Kelima*. Yayasan Bina Pustaka Sarwono, Hal; 64-85.
- [18]. Purnomo SM, 2002. *Pengembangan Obat Tradisional dalam Dunia Pengobatan*. Surabaya: Kanwil Depkes Propinsi Jawa Timur.
- [19]. Sarwono, 1994. *Ilmu Kandungan*. Jakarta: Yayasan Bina Pustaka Sarwono Prawirohardjo, hlm 544-545; 634-643.
- [20]. Speroff L, MD and Phillip D, 1996. *A Clinical Guide for Contraception*, 22nd edition. San Francisco: University of California, pp 35-37.
- [21]. Speroff L, Robert H, and Nathan GK, 2005. *Clinical Embriology Gynecology Endocrinology and Infertility*, 5th edition. Baltimore London: William and Williams Co, pp 76-85.
- [22]. Tambajong J, 1990, *Buku Ajar Histology*, EGC, Jakarta, Hal; 480-509.
- [23]. Verralls S, 2003, *Anatomidan Fisiologi Terapan Dalam Kebidanan*, Edisi 3. Jakarta, EGC, Hal; 161-173.