



The Effect of Cigarette Smoke Exposure to The Number of Implantation Points in Pregnancy Mice (*Mouse muscle*)

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Abstract. Implantation is the process by which the blastocyst sinks into the endometrium after the release of the Zona Pellucida. This study aims to examine the effect of cigarette smoke on the number of implantation points in pregnant mice (*Mouse muscle*). This study used a completely randomized design (CRD), with strained female mice as experimental animals Bal b/c species *Mouse muscle* two months old, totalling 24 tails. Treatment was given on day 1 of pregnancy with control treatment (P0), one cigarette (P1), and two cigarettes (P2). Surgery was performed on the 13th day to calculate the number of implantation points. Data were analyzed using One-Way ANOVA. The results showed the treatment's effect on the number of mice pregnancy implantation points ($p < .05$). Treatment P0 had the highest point of implantation, followed by treatments P1 and P2. These results indicate that cigarette smoke can inhibit the process of cell division, inhibit the formation and development of blastocysts, and further process pre-implantation and implantation in mice.

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INTRODUCTION

Smoking is part of people's lifestyles. The level of education correlates with the level of cigarette consumption. The higher a person's education level, the lower the level of cigarette consumption; the lower a person's education level, the higher the level of cigarette consumption as an active smoker (Sirajuddin *et al.*, 2011). Smoking in Indonesia is one of the highest in the world. It was noted that 46.8% of men and 3.1% of women over ten years were smokers (Salsabila *et al.*, 2022). Tobacco in cigarettes contains more than 4000 elements, and there are about 200 elements that are harmful to health. The main toxins in tobacco are tar, nicotine and CO., Some of the other toxic chemical substances in tobacco include: Hydrogen Cyanide, Ammonia, Toluene, Acetone, Methanol, Naphthalene, Urethane, Vinyl Chloride, Dimethylnitrosamine, Arsenic, Dibenzacridine, Butane, Pyrene Cadmium, Benzopyrene, Naphthylamine, Phenol, Polonium-210, Toluidine and DDT. All these substances are toxic and carcinogenic hazardous to health, and can cause various diseases (Emma *et al.*, 2022).

Smoking can affect the oocyte reduction rate, causing menopause prematurely, inhibiting embryo fragmentation, decreasing sperm quality, and inhibiting the function of the mature



oviduct to enter the uterus (Zenzes, 2000; Gruber et al., 2008). Smoking can also negatively impact fertility. Tobacco compounds impact the ovarian follicle maturation process and the cycle of fertilization parameters in-vitro. In addition, exposure to tobacco compounds impacts pre- and post-implantation embryos (Soares, 2009; Dechanet et al., 2011; Suter & Aagaard, 2020). Smoking can impact pregnant women, which can cause premature birth, low baby weight, prenatal mortality or intrauterine death rates, babies born with disabilities, and babies born with developmental disorders (Xu et al., 2017; McDonnell & Regan, 2019). Smoking during pregnancy increases the incidence of cleft lip and palate in infants caused by hypoxic conditions of the embryonic tissue (Lacerda et al., 2021; Fell et al., 2022). Research that examines the effect of cigarette smoke on the point of implantation in pregnant mice is lacking. Therefore, this paper describes the results of testing the effect of cigarette smoke on the number of implantation points in mice.

RESEARCH METHODS

Experimental Animals

The experimental animals used in the study were mice (*Mouse muscle*) strain *Balb/c*, with female gender aged two months weighing 20-40 grams. There were 24 mice used, maintained in a plastic cage with room temperature between 25-30°C, quiet place, quiet atmosphere, somewhat dim and not too bright.

Mice Mating (*Mouse muscle*)

Mating in mice was carried out by mixing one male mouse and two female mice in 1 cage. Mixing was carried out for 12 hours from 5 pm to 5 am. Proof of mating mice was taken from the presence of vaginal plugs (*vaginal plugs*), a lump of fluid (sperm residue) covering the vaginal opening, found the next day after mixing. The presence of a vaginal plug determines the 0th day of gestation of the mice.

Mice Treatment (*Mouse muscle*)

Mice declared 0th pregnant were then given and displayed cigarette smoke on the first day of pregnancy. Exposure was carried out according to the treatment group P0 as a control (without giving cigarettes), P1 (1 cigarette), and P2 (2 cigarettes). The treatment time for each cigarette is 10-15 minutes. Treatment was given every day until the 13th day.

Mice Surgery (*Mouse muscle*)

Surgery is done on the day of the final treatment. Retrieval of uterine organs to calculate the number of implantation points is carried out by the method of dislocation (Hidayat & Wulandari, 2021). Cutting was done by cutting mice's ovaries first, then by cutting the uterine organs by cutting the fat and blood vessels and the uterus's base (Deb et al., 2006).



Data analysis

The number of implantation points was surgically assessed on the 13th day of gestation. Further research data were analyzed using One-Way ANOVA (Heiberger et al., 2009) with a 95% degree of confidence to determine the effect of each treatment on the point of implantation in mice.

RESULTS AND DISCUSSION

Number of Implantation Points

Data on the average number of implantation points of mice (*Mouse muscle*) in the control treatment P0 totalled nine implantation points, P1 averaged six implantation points, and P2 averaged four implantation points. The results of calculating the number of implantation points for each treatment, P0, P1, and P2, were then presented to determine the percentage success ratio of the number of implantation points for each treatment. The percentage of the number of implantation points for each treatment is presented in Figure 1.

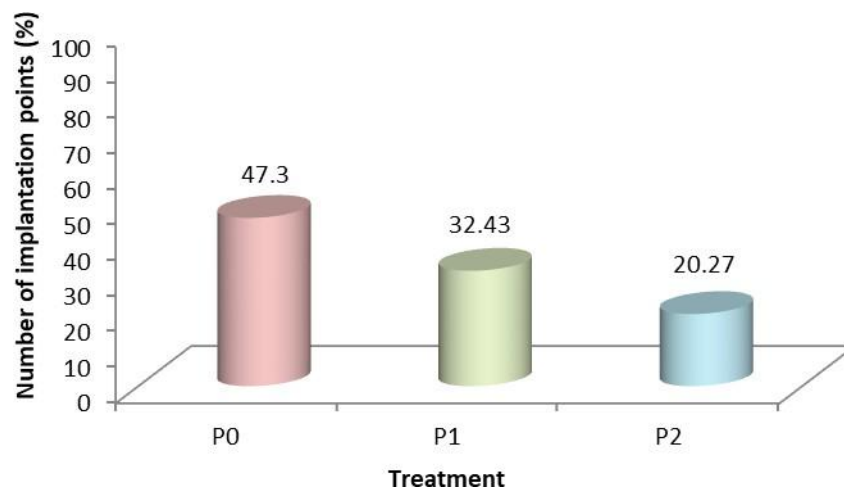


Figure 1. Histogram of the Average Number of Implantation Points in Mice (*Mouse muscle*) P0 (control), P1 (1 cigarette), P2 (2 cigarettes).

Figure 1 shows that the highest average number of implantation points was in P0 with the control treatment, namely 47.30%, while the lowest was in P2 with two cigarette treatments at 20.27%. Analysis results in one-way ANOVA to determine the effective treatment on the number of implantation points show that value *p value* < 0.05. Therefore, treatment significantly affects the number of implantation points in mice (*Mouse muscle*). The highest number of implantation points was found in treatment P0, followed by P1 and P2. The number of P0 treatment implantation points is shown in Figure 2.



Figure 2. Number of Implantation Points in P0 Treatment (Control) in Mice (*Mouse muscle*).
Picture a) P0U2, b) P0U4, and c) P0U5.

One cigarette obtained the second-highest number of implantation points in the P1 treatment. The average number of implantation points in this treatment was 6 points. These results were lower than in treatment P0. P1 treatment implantation points are displayed in Figure 3.

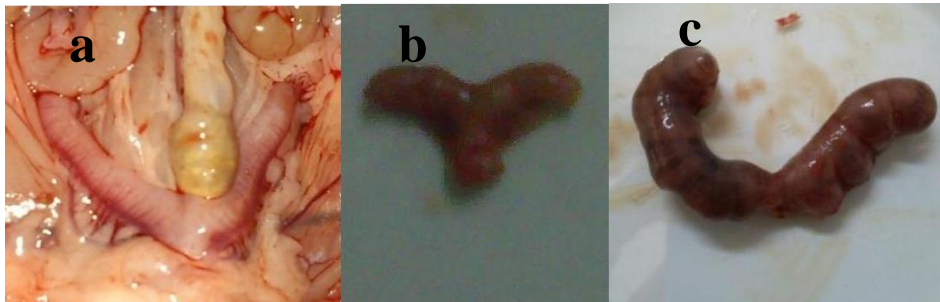


Figure 3 Number of Implantation Points in treatment P1 (1 cigarette). Figure a) P1U3, b) P1U4, and c) P1U7.

The P2 treatment (2 cigarettes) showed fewer implantation points than the other two treatments, with an average number of four implantation points. The implantation point in the P2 treatment is shown in Figure 4.

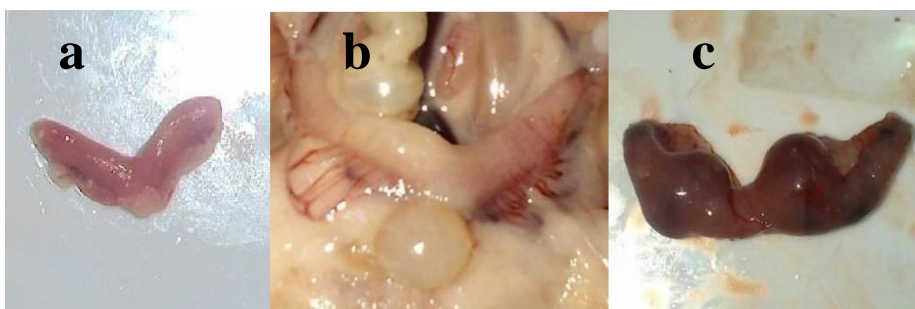


Figure 4 Number of Implantation Points at P2 (2 cigarettes). Figure a) P2U2, b) P2U6, and c) P2U8.

The results showed that treatment P1 and P2 white display cigarette smoke impacts the number of implantation points in mice. Cigarette smoke can inhibit cell division, the formation and development of blastocysts, and ultimately bother pre-implantation and implantation



processes. This study's results align with Samsuria (2009), which states that an implantation point's absence characterizes implantation failure in an extended right or left uterus. Implantation failure can occur early in the implantation process, where the zygote has been fertilized but cannot reach the uterus.

Figures 3 and 4 show that implantation failure due to cigarette smoke chemical compounds inhibits the process of cell division, inhibiting the formation of elastosis so that the fertilized zygote cannot reach the uterus. It is evidenced by blood vessels supporting the uterus, indicating that fertilization has occurred. According to Zenzes (2000), the compound benzo[a]pyrene is a polycyclic hydrocarbon (PAH) in cigarette smoke which can bind covalently with DNA, causing adduct, and is mutagenic and carcinogenic. *Adducting BPDE-DNA* in smokers' ovarian cells can result in primordial oocyte damage to the embryo pre-implantation.

The ovum is surrounded by a glycoprotein membrane called the zona pellucida. After fertilization, the zona pellucida becomes hard. Along with the growth of the pre-implantation embryo, the blastocoel will increase in size and become a blastocyst. In mice, the blastocyst is formed on the fourth day and will implant on the fifth day after fertilization. The zona pellucida becomes thin and elastic due to the fluid in the blastocoel. The chemical compounds of cigarette smoke in the P1 and P2 treatments caused the zona pellucida to thicken. According to Shiloh *et al* (2004), the thickness of the zona pellucida of oocytes and embryos in active and passive smokers increases compared to non-smokers' zona pellucida. Zenzes (2000) suggests stress oxidative Cigarette smoke causes chromosomal errors in meiosis. Implantation failure is thought to have occurred on the fifth day of pre-implantation due to oocyte damage due to incomplete division and blastocysts that cannot hatch from the zona pellucida so that blastocysts cannot attach to the uterine wall.

The low point of implantation in the P1 and P2 treatments is thought to be due to a substance in cigarette smoke. The nicotine effect contained in Cigarette smoke in pregnancy can develop oxidative stress, which can inhibit blastocyst adhesion, migration, and invasion required for intrauterine implantation (Kamsani *et al.*, 2012). Shao *et al.* (2012) stated that nicotine in cigarette chemical compounds could slow down the entry of the embryo into the uterus, impair dehydration, and delay intrauterine implantation. Embryo implantation aberrant due to cigarette smoke compounds can cause ectopic pregnancy (EP).

CONCLUSION

Based on the study's results, it can be concluded that cigarette smoke can inhibit the process of cell division, inhibit the formation and development of blastocysts, and interfere with the pre-implantation and implantation processes in mice, with a significance value of $p < 0.05$. Treatment P0 had the highest point of implantation, followed by treatments P1 and P2.

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