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Effect of sterilization of female and male rats on osteocyte cells

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Abstract

Sterilization is the recommended method of conception for animals. This method has also been used in many countries to control populations in dogs and cats. However, this action has some long-term effects as it affects the production of reproductive hormones in the body. This study aims to determine the effect of sterilization on the number of osteocytes. This study is an experimental study with a Posttest-Only Control Design, by observing rats that were fed standard feed until the age of 16 weeks and then terminated and measured the planned variables. A total of 24 rats aged 10 weeks were divided into 4 groups, namely 6 control females, 6 ovariohysterectomy females, 6 orchiectomy males, and 6 control males. 6 weeks after sterilization, all rats were euthanized and the left thigh bone was taken for histological examination of bone cells, namely osteocytic cells. The results of the analysis using the independent t-test, the results of the osteocyte cell test in females were $p<0.05$ which showed statistically that the sterilization action in female rats had a significant effect on the number of osteocyte cells, while in male rats, the value of $p>0.05$, which means statistically sterilization measures. had no significant effect on the number of osteocytes.

Keywords: Ovariohisterectomy, Sterilization, Osteocytes Cells

Introduction

Sterilization is the recommended method of conception for animals. There are several methods of sterilization that can be carried out, including surgical procedures and hormonally, but the recommended population control program is through surgical procedures (OIE, 2019). Sterilization by inhibiting hormones is only temporary and does not only affect target organs but can damage other organs as well (Basa and Ibrahim, 2019). Long-term use of hormonal contraceptive methods has been shown in several studies to cause severe side effects such as endometriosis, pyometra, fibrinous-cystic hyperplasia, mammary tumors, and ovarian cysts (Vasetska and Mass, 2017). In addition, based on the latest research, 60.6% of respondents

think that sterilization with surgical procedures is the most appropriate action for population control (Hanif et al. 2017). This method has also been used in many countries to control populations in dogs and cats. Another advantage that can be obtained through this method compared to hormonal contraception is that it can reduce the incidence of diseases in animals such as metritis, mastitis, dystocia, vaginal prolapse, to mammary hypertrophy (Kustritz, 2012). However, the long-term effects of sterilization are still not clearly understood (White, 2020).

Orchidectomy and ovariohysterectomy is the removal of the main tool in the reproductive organs. Where in females is called ovariohysterectomy, which is the removal of the ovaries and uterus, while orchidectomy in males is removal of the testes. As is known, the two main reproductive organs are the main producers of reproductive hormones in the body. Organs that produce the hormone estrogen include the ovaries, adrenal cortex, and adipocyte cells which in these organs will produce estrogen when the ovaries do not produce estrogen (Nelson, 2001). While the testes are the producers of the hormone testosterone which is an androgen which is synthesized 95% in the testes and the rest in the adrenals (Laswati, 2016). By knowing that the ovaries and testes are the main producers of estrogen and androgen hormones in the body, when an ovariohysterectomy and an orchidectomy are performed, the levels of these two hormones in the body will decrease. In addition to estrogen and androgen hormones that function in the formation of primary sex organs, these two hormones are also very important in maintaining bone homeostasis (Laswati, 2016). The effects of estrogen and androgens are only seen in cells and tissues that have estrogen receptors, including bone cells. Therefore, in this study, we will calculate the number of osteocyte cells between sterilized rats compared to control rats.

Materials and Methods

Twenty-four male and female wistar rats were used in this study. Mice were 10 weeks old and weighed 200 to 250 grams. Rats were placed 1 to 2 each in one cage. All rats were given standard feed with feed composition, namely water: maximum 12%, crude protein: minimum 15%, crude fat: 3-7%, crude fiber: maximum 6%, ash: maximum 7%, calcium: 0.9-1 ,1% and phosphorus: 0.6-0.9% and the provision of drinking water is not limited. Rats were divided into 4 groups, namely control female group, ovariohysterectomy female group, control male group and orchiectomy male group. Ovariohysterectomy was performed transabdominally and orchiectomy was performed with the scrotal. Mice were reared 6 weeks after surgery. Then blood was taken to test calcium levels and followed by euthanasia using ketamine anesthesia for taking the os femur for making histological preparations to count the number of osteocytes.

Female rats were prepared by shearing at the surgical site, namely the ventral abdomen and then prepared aseptically using povidone iodine. Total anesthesia was performed using intramuscular injection of ketamine and xylazine. An incision was made on the medial part of the abdomen for 2 cm starting from the skin, subcutaneous, and muscle, then the abdomen was explored to get the ovaries. Ligation is performed on the ovarian vessels and between the uterine cervix and uterine corpus, then the ovaries and uterus are removed. Before the suturing process, the abdomen is given antibiotics first, then each layer that has been cut, is sewn back using absorbable threads and on the skin using non-absorbable threads. Antibiotics are given postoperatively to prevent infection.

Male rats were prepared by shaving at the surgical site, namely the scrotum and then prepared aseptically using povidone iodine. Total anesthesia was performed using intramuscular injection of ketamine and xylazine. The incision was made right in the scrotum for 1 cm starting from the skin, tunica dartos, tunica vaginalis visceralis et parietalis, to the tunica albuginea, then the testes were removed from the scrotum. Ligation is performed on the testicular vessels, then the testes

are removed. Before the suturing process, the scrotum is given antibiotics first, then the skin is sutured using non-absorbable thread. Antibiotics are given postoperatively to prevent infection.

Results and Discussion

The examination of the number of osteocytes was carried out by histological examination with Hematoxylin Eosin staining, with a magnification of 40X10 and then the number of osteocytes was counted.

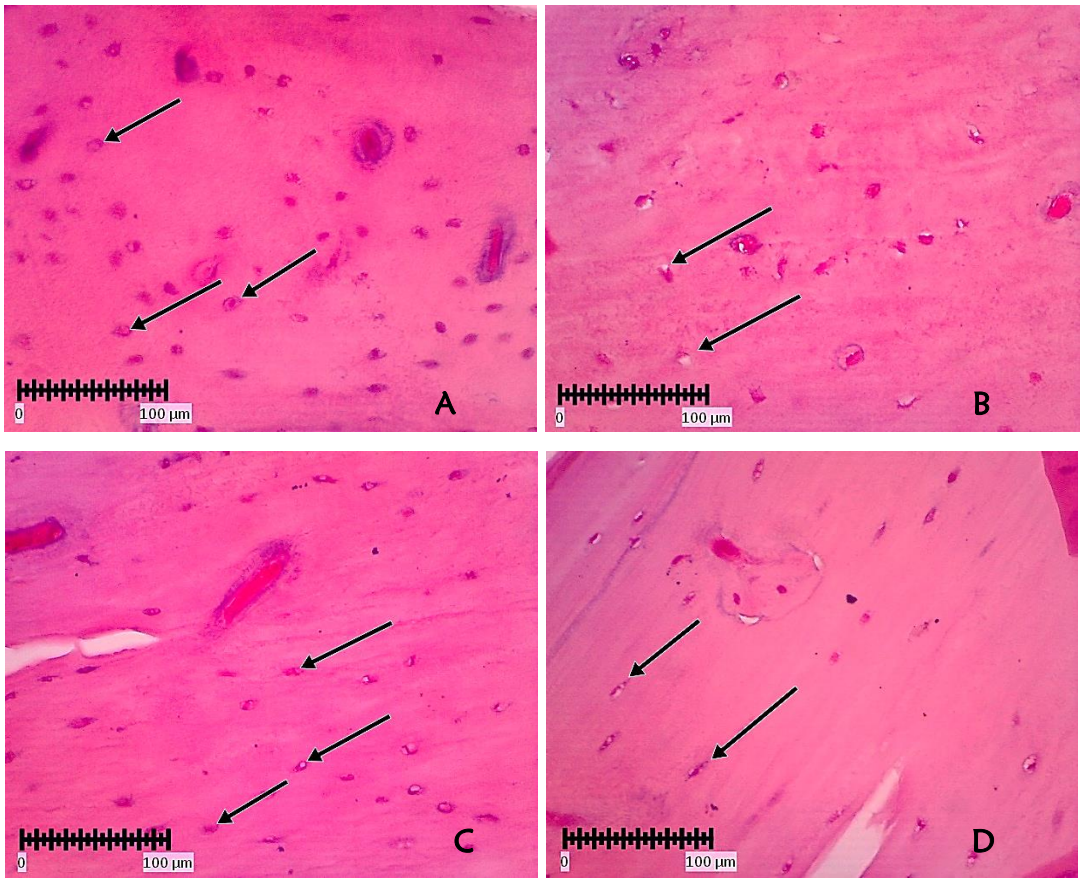

Figure 1. Histology of female control (A), histology of female treatment (B), histology of male control (C), histology of male treatment (D), osteocyte cells in bone (Black arrows). Hematoxylin eosin staining. I Bar: 100 µm.

Table 1. Results of Descriptive Analysis of Osteocyte Cell Number Data in Control and Treatment Female Groups

Female Rat	n	Average Osteocyte	*p
Control	6	30.17 ± 4.79	0.024
Treatment	6	23.83 ± 3.31	

*p= Independent t test

Table 2. Results of Descriptive Analysis of Osteocyte Cell Number Data in the Control and Treatment Male Group

Male Rats	n	Average Osteocyte	*p
Control	6	30.17 ± 6.17	0.069
Treatment	6	24.33 ± 3.32	

**p= Independent t test*

To determine the relationship between the number of osteocytes and the sterilization procedure, we divided the data processing into 2 groups. The first is the female ovariohysterectomy group and the female control group. The results of data analysis showed the mean value of the control group was 30.17±4.79 and the treatment group was 23.83±3.31. when viewed from the mean value of the number of osteocyte cells in control females were more than in ovariohysterectomy females. Then we continued with the independent t test to determine the relationship between ovariohysterectomy and the number of osteoclast cells. The test results showed a value of $p<0.05$, namely $p=0.024$, it showed that statistically there was a relationship between sterilization and the number of osteocyte cells in female rats.

Organs that produce the hormone estrogen include the ovaries, adrenal cortex, and adipocyte cells which in these organs will produce estrogen when the ovaries do not produce estrogen (Nelson, 2001). The ovariectomy process can reduce estrogen levels, this is because the ovaries as the main producer of the estrogen hormone do not function, so that the estrogen levels in ovariectomized rats will decrease drastically (Karaman et al., 2013).

In vitro studies have found that estrogen receptors and translate mechanical forces into pro-survival signals in osteocytes and osteoblasts. Estradiol prevents the occurrence of apoptosis in osteocytes and increases the production of TGF which will inhibit bone resorption by osteoclasts (Aguirre et al., 2007).

At the tissue level, estrogen reduces bone turnover, the emergence of osteocytes that regulate bone remodeling activation, possibly that the antiremodeling effect of estrogen is mediated through these osteocytes. Osteocytes are cells in the bone matrix, derived from osteoblasts that help in the process of bone remodeling. The overlying bone cells comprise a subpopulation of the osteoblast family. These cells are attached to the surface of the bone where there is collagen. The bone cell layer migrates to form a roof/scaffold especially in areas close to the osteoclasts. Estrogen deficiency is associated with increased osteocyte apoptosis in humans (Kilic TO., 2015).

By looking at the function that in osteocyte cells there are receptors of estrogen, then when there is a deficiency of the hormone estrogen this can make osteocyte cells undergo apoptosis so that their number can decrease according to the results of data analysis based on histology with a magnification of 40X in Figure 1 A (control female) and Figure 1 B (Female treatment) found the number of osteocyte cells in the treated female was less than the number of osteocyte cells in the control female. The results of the independent t test also showed a p value <0.05, which means that statistically there is a relationship between sterilization and the number of osteocytes in female rats.

In the second group, there were orchiectomy males and control males. From the results of data analysis, the mean number of osteocytes in male control rats was 30.17±6.17 and the average value in orchiectomy rats was 24.33±3.32. when viewed from the mean value of the number of osteocyte cells in the treatment male was lower than the number of osteocyte cells in the control male. However, after continuing the independent t test, the value of $p> 0.05$ was $p = 0.06$. This shows that statistically there is no correlation between sterilization measures and the

number of osteocytes, although there was a decrease in the number of osteocytes in the sterilization group compared to the male control group.

The most important circulating androgen hormone in men, is secreted 95% by the testes and the rest by the adrenals (Laswati, 2016). Estrogen in males is produced by aromatization of androgens through the aromatase enzyme, a cytochrome P450 enzyme encoded by the CYP19A1 gene. This aromatase enzyme catalyzes the conversion of 4-androstenedione to estrone and T to E2. Aromatase is found in testicular tissue (Sertoli and Leydig cells) (Laswati, 2016).

Bone is an endocrine tissue that expresses androgen receptors (RA), REα and REβ, which are sensitive to androgens and estrogens. These receptors are expressed in osteoblasts, osteoclasts, osteocytes and chondrocytes in the epiphysis (Laswati, 2016). High RA expression in osteocytes, as it is known that osteocytes are present in the highest number in bone, plays an important role in the regulation of bone remodeling, as well as in response to mechanical loading. Osteocytes express receptor activator of NF-κB ligand (RANKL) which plays a role in bone remodeling and is regulated by androgens. It is suspected that in the aging process, RA inactivation occurs in osteocytes which causes a decrease in the integrity of the trabecular bone (Laswati, 2016).

The effect of orchiectomy will reduce levels of androgen hormone and estrogen hormone in the rat body which will affect bone osteocyte cells because osteocyte cells have androgen receptors and estrogen receptors. The insignificance of the results of data analysis on the number of osteocyte cells could be due to the limited time in the study and the high number of osteocyte cells in one of the orchiectomy rats samples. This can be caused by external variables that cannot be controlled, such as the psychological condition of the rat and the initial condition of the rat before being given treatment. Although the statistical test results are not significant, we can see in the histopathological picture with a magnification of 40X in Figures 1 C (control males) and 1 D (treated males) the number of osteocyte cells in treated males is less than in control males and the average value of the number of osteocyte cells in males' treatment was lower than the control male.

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