Jurnal Riset Veteriner Indonesia
Journal of the Indonesian Veterinary Research
P-ISSN: 2614-0187, E-ISSN:2615-2835
Volume 6 No. 1 (January 2022), pp. 32-39
journal.unhas.ac.id/index.php/jrvi/

The Effect of Sterilization on Vitamin D Levels in Male and Female Rats

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Abstract

Sterilization is an operation that can prevent reproduction and is useful as a treatment. Sterilization is done by removing the reproductive organs, in males it is called orchiectomy and in females it is called ovariohysterectomy which causes a decrease in the production of reproductive hormones. Previous studies have proven a physiologic relationship between reproductive hormones and vitamin D activation, but there is no information on the effect of sterilization on vitamin D levels. This study aimed to determine the effect of sterilization on vitamin D levels. This study was experimental with a Posttest-Only Control Group design. design. A total of 24 wistar rats consisting of 12 male rats and 12 female rats and each were divided into two groups, 6 female rats sterilized (ovariohysterectomy), 6 female rats non-sterilized, 6 male rats sterilized (orchiectomy) and 6 male rats non-sterilized. -sterilization. Wistar rats that were 10 weeks old were transferred to their respective cages and given regular feed to all groups of rats for 7 days as an adaptation process, after which sterilization was carried out in the sterilization group. After 6 weeks post-treatment, the rats were 17 weeks old and then blood was taken for examination of Vitamin D levels using the elisa method which was carried out at Hum-Rc Hasanuddin University Hospital. The results showed that the mean value of vitamin D levels in the ovariohysterectomy group was lower than in the non-sterilized group and was significant (Mann-Withney, $p<0.05$). The orchiectomy group was also lower than in the non-sterlized group and not significant (Independent T test, $p> 0.05$). It can be concluded that sterilization can reduce vitamin D levels in pets.

Keywords: *Sterilization, Ovariohysterectomy, Orchiectomy, and Vitamin D*

Introduction

Sterilization is an operation that can prevent reproduction and is useful as a treatment (Yuliana, Wiryanthini, Karmaya, & Widarsa, 2012). One of the reasons for sterilization is that there is a large increase in the population of pets that can threaten human health, pets can transmit various disease agents, one of the causes of uncontrolled population is unwanted marriage. (Root Kustritz, 2012). Sterilization is distinguished between male animals (orchiectomy) and female animals (ovariohisterectomy) (Moldave & Rhodes, 2013).

The sterilization process reduces estrogen hormone levels because the ovaries as the main producer of the estrogen hormone are no longer functioning, so that the estrogen levels in sterilized females will decrease (Yuliana et al., 2012). Likewise in male animals there will be a decrease in androgen hormones which are produced by the testes. Estrogen plays a role in the process of homeostasis, one of which can increase levels of 1,25 dihydroxycholecalciferol which serves to increase the degree of calcium absorption in the intestine so that it affects bone anabolic (Nelson & Bulun, 2001). Estrogen functions to stimulate the formation of 1,25 DHCC (vitamin D3) in the kidneys (Calvo & Park, 1996). Androgens/testosterone increase 1-a-hydroxylase, a key enzyme in vitamin D metabolism that converts 25(OH)D to 1,25(OH)2D (De Toni et al., 2014), and It has been demonstrated that the male reproductive tract expresses most of the enzymes involved in vitamin D, activation via 25-hydroxylation, testes and epithelium, ejaculatory ducts capable of metabolizing vitamin D and expression of VDR metabolic enzymes, present in germ cells during spermatogenesis (Blomberg Jensen et al., 2010).

Vitamin D is an essential fat-soluble nutrient that plays a number of important functions in the body to maintain health and prevent disease (Tiwari & Sharma, 2017). Vitamin D is naturally found in 2 forms: D2 (ergocalciferol) and D3 (cholecalciferol); Of the two, vitamin D3 is the form that our skin produces after exposure to sunlight. Both forms of vitamin D are considered prohormone and biologically inactive: Vitamin D must undergo two transformation steps in the body before it is activated and carries out its biological function (Hanwell, 2016).

The first step of Vitamin D3 activation occurs in the liver with the addition of a pair of atoms for vitamin D to become 25-hydroxyvitamin D3, or 25(OH) D3. The concentration of 25(OH)D3 in the blood persists for a long time. The second step occurs in the kidney, the LDL receptor, namely magnalin, plays an important role in the internalization of endocytic 25(OH)D3. In the proximal renal tubule 25(OH)D3 is hydroxylated at the carbon 1 position of ring A, producing the active hormone of vitamin D, 1,25-dihydroxy vitamin D3 (1,25(OH)2D3) (also called calcitriol) which is biologically active, a form of hormone that is responsible for most of the physiological mechanisms. This form does not last long in the blood before use (Hanwell, 2016). On this basis, the researchers wanted to see vitamin D levels in sterilized animals. This study aims to determine the effect of sterilization in male and female rats on vitamin D levels.

Meterials and Methods

This study is an experimental study with a Posttest-Only Control Design, 24 Wistar rats aged 10 weeks with healthy physical characteristics and active movement obtained from the Animal Clinic Education, Hasanuddin University, Veterinary Professional Education, Faculty of Medicine, Hasanuddin University Makassar wistar consisted of 12 male rats and 12 female rats, each of which was divided into two groups, namely the case group and the control group.
The rats were transferred to their respective cages and given regular feed to the whole group of rats for 7 days as an adaptation process. In the case group, sterilization was performed, male rats were performed orchiectomy and female rats were subjected to ovariohisterectomy. After 6 weeks post-treatment, the rats were 16 weeks old and then the kidneys were taken to check the levels of Vitamin D. This study has been approved by the ethics committee of the Faculty of Medicine, Hasanuddin University with the number 68/UN4.6.4.5.31/PP36/2021.

Orchiectomy

Case animals were fasted 8-12 hours before surgery, animals were under general anesthesia using a combination of xylazine and ketamine. Prior to the administration of anesthesia,

atropine sulfate premedication was administered subcutaneously and 10 minutes later induced by a combination of xylazine and ketamine, each intramuscularly.
Removal of the testicles with open castration technique, an incision is made in the skin area of the scrotum and then the tunica vaginalis and tunica albuginea are cut, the testes are removed from their sheathing, the vas deferens is pulled and separated by blood vessels, the blood vessels are clamped with hemostat forceps, incisions and ligations are performed. on blood vessels Work steps 1-8 are performed on both testes. The skin was sutured using a simple interrupted method. Betadine was given to the incision area after orchiectomy. Postoperative care is given with antibiotics and analgesics to prevent infection and reduce pain.

Ovariohysterectomy

Case animals were fasted 8-12 hours before surgery. Animals are under general anesthesia using a combination of xylazine and ketamine. Prior to the administration of anesthesia, atropine sulfate was premedicated subcutaneously and 10 minutes later induced by a combination of xylazine and ketamine, each intramuscularly, after the animal was prepared, the case was placed on dorsal recumbency, and the ventral abdomen was prepared as the operating area.
The umbilicus was identified and thought to divide the abdominal area into 3 parts. The animal cases were shaved from the umbilicus to the caudal area and then disinfected using 70% alcohol and iodine, the incision was made from the caudal umbilicus 1/3 of the cranial abdominal to the caudal about 4-8 cm. An incision is made in the skin and subcutaneously to open the linea alba. The linea alba is held and lifted slightly out to allow an incision in the linea alba to open the abdominal cavity. The left abdominal wall was exposed and the uterus was explored. To confirm that the uterus is being removed, it is traced caudally to locate uterine biforcations and cranially to locate the ovaries. Once the ovary is found, palpate for the presence of the suspensary ligament at the proximal end of the ovary. Traced and performed suspensary ligament severance so that the ovary can be removed. Ligation was performed using 2 arterial clamps near the ovary. The most proximal (inside) clamp was used for ligation and ligation using absorbable PGA sutures. Then cut between the two arterial clamps and control the occurrence of bleeding.
The ovary is removed and the hanger is cut in a section with minimal blood vessels. The same procedure was performed on the second ovary. The abdominal wall was closed and sutured with three layers, namely the linea alba and peritoneum with interrupted suture patterns, subcutaneous and fascia with continuous patterns and skin with interrupted patterns. Postoperative care is given with antibiotics and analgesics to prevent infection and reduce pain. Suture wounds on the abdomen were given bioplacenthon ointment 2 times a day and closed using hypafix.

Vitamin D Level Check

Vitamin D levels were measured using the ELISA technique. Prior to the elisa technique, the kidney organs were extracted first. The reagent used is Rat Vitamin D3 ELISA Kit with catalog number E0615Ra.

The basic principle of the ELISA method is the use of enzymes to detect the bond between antigen and antibody, which changes the color from blue to yellow and the color intensity is measured at 450 nm using a spectrophotometer for 10 minutes, to measure the concentration of vitamin levels in the sample. The spectrophotometer is connected to a computer so that vitamin D levels will be read and concentration data is stored in excel form.

Data Analysis

The data were processed by SPSS version 22 program. The analytical test used in this study was the *independent t test* and the *Mann-Whitney test* and were considered significant if the value of $p<0.05$ was reached.

Results and Discussion

Effect of Ovariohysterectomy on Kidney Vitamin D Levels

The results showed that the significant value of vitamin D levels using the *Mann-Whitney* test in the ovariohysterectomy and non-sterilized female groups was (p) <0.05, so it can be concluded that there was a significant difference between the ovariohysterectomy and non-sterilized groups in female rats. The mean levels of vitamin D in the non-sterilized group were 9,740 ng/ml higher than in the ovariohysterectomy group. The difference in vitamin D levels in the ovariohysterectomy group and the control group was due to changes in estrogen hormone levels in female rats, where when ovariohysterectomy was performed, estrogen production decreased (Ibrahim & Zaid, 2017).

Table 1. Differences in vitamin D levels of female rats in the ovariohysterectomy and non-sterilization groups

Female rats	n	Mean Vitamin D Levels (ng/ml) ± SD	**p*
Non- sterilization	6	17,970 ± 2,005	0.037
Ovariohysterectomy	6	8,230 ± 3,639	

**p= Mann-Withney test*

Estrogen can stimulate vitamin D metabolism by increasing 1,25(OH)2D in postmenopausal women (reduced estrogen in the body) (Huang et al., 2019), from research Huang proves that by giving estrogen to postmenopausal women can increase 1.25 (OH) 2d. Exogenous estrogen has been associated with an increase in vitamin D-binding protein (VDBP), the metabolically active metabolite 1,25(OH)2D and (OH) vitamin D(3) (Harmon, Umbach, & Baird, 2016). Estrogen directly stimulates calcitriol production by modulating lα-hydroxylase. activity (Gallagher, Riggs, & Deluca, 1980). Renal activity of 1αOHase is known to be under the control of several factors, some of which are hormonal, the main endocrine modulators of 1,25(OH)2D synthesis are parathyroid hormone and estrogen (Huang et al., 2019).

Female rats with ovariohysterectomy greatly affect the work of the kidneys in activating vitamin D. Homeostasis of vitamin D is very important to control calcium and phosphate balance in health. The circulating level of the active form of vitamin D, 1.25 dihydroxyvitamin D (1.25(OH)2D) is largely determined by 1.25(OH)2D production in the kidney, the circulating concentration of 1.25(OH)2D mainly reflecting its synthesis. in the proximal tubule of the kidney (Vitamin D and Kidney Disease), where a second hydroxyl group (1α-hydroxylase) is added to convert it to 1 alpha, 25-hydroxyvitamin D, or 1,25(OH)2D (also called calcitriol) which It is a biologically active form of the hormone (Hanwell, 2016). 1α-hydroxylase is the bioactivation of vitamin D, and the activity of this enzyme is tightly regulated in the kidney by PTH, calcium, phosphorus, 1,25(OH)2D (Portale, Perwad, & Miller, 2012). As has been explained by the action of ovariohysterectomy which causes a decrease or even loss of the hormone estrogen can trigger a decrease in the production of 1α-hydroxylase as the main enzyme in the activation of vitamin D in the kidneys. Estrogen directly stimulates calcitriol production by modulating 1α-hydroxylase. activity (Cheema, Grant, & Marcus, 1989). Several previous studies have shown that physiologically estrogen greatly affects the metabolism of vitamin D in the kidneys.

Research conducted by (Ritterhouse et al., 2014) Using detailed information from 1662 young African-American women in the Detroit area, the results provide strong evidence to support the hypothesis that the use of exogenous estrogens increases serum 25(OH)D, as well as the study (Møller et al., 2013) identified a 20% independent increase in 25(OH)D among women using estrogen-containing contraceptives.

Effect of Orchiectomy on Kidney Vitamin D Levels

Table 2 shows that the significant value of Vitamin D levels using the Independent t test in the orchiectomy and non-sterilized male groups was (p)> 0.05, so it can be concluded that there is no significant difference between orchiectomy and non-sterilization. The average vitamin D level in the non-sterilized group was 0.605 ng/ml higher than the orchiectomy male group. Although it was not statistically significant, it was seen from the mean that the sterilization group was lower than the control group. As in female rats undergoing ovariohysterectomy, reproductive hormones will no longer be produced, so that in male rats the same thing happens where the androgen hormone will be lost. The effect of an orchiectomy is that testosterone levels will decrease, testosterone is directly associated with androgen receptor density so that the absence of testosterone (orchiectomy) is associated with a decrease in androgen receptors in the body (Shortliffe, Ye, Behr, & Wang, 2014).

Table 2. Differences in vitamin D levels of male rats in the orchiectomy and non-sterilization groups

Male rats		Mean Vitamin D Levels (ng/ml) ± SD	*p
Non-sterilization	6	10,126 ± 0,987	0.411
Orchiectomy		9,521 ± 1,314	

**p= Independent T test*

It has been shown that androgens increase 1a-hydroxylase, a key enzyme in vitamin D metabolism that converts 25(OH)D to 1,25(OH)2D (De Toni et al., 2014) it has been demonstrated that the male reproductive tract expresses most of the enzymes involved in vitamin D, activation via 25-hydroxylation, testes and epithelium, the ejaculatory tract is able to metabolize vitamin D, Expression of metabolic enzymes of VDR, CYP2R1, CYP27A1, CYP27B1 and CYP24A1 simultaneously expressed in the testes, epididymis, prostate, and seminal vesicles (Blomberg Jensen et al., 2010). Data from human and animal models clearly show that CYP2R1 (microsomal vitamin D 25-hydrosylase) secreted in testicular Leydig cells is the main enzyme involved in Vit D 25-hydroxylation, and its expression is under LH control. According to this model, clinical conditions with musculoskeletal damage are often associated with decreased serum 25-hydroxy Vit D (25-OH Vit D) levels along with elevated serum PTH (C. Foresta et al., 2013). Research conducted by (Wang et al., 2010) shows that bilateral orchiectomy resulted in 33% of cases decreased levels of 25 (OH) D even though there is an adequate testosterone replacement therapy. Expression of vitamin D receptors and vitamin D metabolizing enzymes in human testes (Blomberg Jensen et al., 2010) or impaired renal 1αhydroxylase activity due to low testosterone levels (Francis et al., 1986) shows a close functional relationship between testosterone and vitamin D.

The insignificance of the results obtained may be due to existing references that testes and ejaculatory duct epithelium are capable of metabolizing vitamin D, the distal epididymis, seminal vesicles and prostate all express vitamin D receptor metabolizing enzymes CYP2R1, CYP27A1, CYP27B1 and CYP24A1 (Blomberg Jensen et al., 2010). in this case the male reproductive organs can produce enzymes that play a role in activating vitamin D. It's just that these enzymes are not only produced in the testes, but CYP is widespread in the body (Bikle, 2014). CYP27B1 have high levels of homology with other mitochondrial enzymes involved with vitamin D metabolism: CYP27A1 and CYP24A1. Although the kidney is the primary source of circulating 1,25(OH)2D, a number of other tissues also express the enzyme, and regulation of extrarenal CYP27B1 differs from that of renal CYP27B1 (Adams & Hewison, 2012). CYP2R1 appears to be the main 25-hydroxylase, but other enzymes have 25-hydroxylase activity that can affect 25OHD levels in certain tissues and/or contribute to circulating 25OHD levels (Bikle, 2014). therefore, researchers assumed by the loss of the testicles so do not affect the levels of vitamin D so that the decline is not drastic as in females ovariohisterectomy.

From this study it can be concluded that sterilization can affect vitamin D levels, Vitamin D also has a significant effect on the immune system (Gruber, 2015). Vitamin D has many physiological roles, Its main function is to maintain calcium and phosphorus homeostasis, which is necessary for proper bone mineralization (Zadka, Pałkowska-Goździk, & Rosołowska-Huszcz, 2018). Vitamin D increases the ability of small intestinal epithelial cells to absorb calcium and regulates the absorption of phosphorus from food, and also stimulates re-adsorption of calcium from the glomerular filtrate (Christakos, Dhawan, Verstuyf, Verlinden, & Carmeliet, 2015), So the importance of providing intake of vitamin D levels in animals that have been given sterilization measures in this case ovariohysterectomy and orchiectomy.

Conclusion

The mean vitamin D in the sterilized group in females was lower than the mean vitamin D in the non-sterilized group and significant results were obtained. In sterilized males the mean vitamin D was lower than the non-sterilized group and the results were not significant.

Acknowledgment

The author thanks to the Ministry of Research and Technology/BRIN for the master thesis grant No. 7/AMD/E1/KP.PTNBH/2020. The author states there is no conflict of interest with the parties concerned in this study.

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