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Effect of castration and vasectomy on some oxidative stress parameters and blood hormone levels in rats

[*Efeito da castração e da vasectomia em alguns parâmetros de estresse oxidativo e níveis de hormônios no sangue*]

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ABSTRACT

Vasectomy and castration are the most preferred surgical methods to control reproduction in males. While sexual functions are terminated reversibly in vasectomy, they are removed irreversibly in castration. After these processes, changes are observed in hormones and oxidative stress parameters. In this study, we investigated the effects of vasectomy and castration operations on blood follicle stimulating hormone (FSH), luteinizing hormone (LH), testosterone, nitric oxide (NO) and malondialdehyde (MDA) levels in rats. As a result of the analysis, it was determined that FSH, LH, NO, and MDA levels increased ($p<0.05$) and testosterone levels decreased ($p<0.05$) in the castration group compared to the sham and vasectomy groups. Considering the data obtained from the present study, when the two operations (vasectomy and castration) are compared in rats, which are preferred for the control of reproduction, it is thought that vasectomy is a healthier method because it is reversible, does not affect hormone levels, and does not increase oxidative stress.

Keywords: castration, hormones, oxidative stress, vasectomy

RESUMO

A vasectomia e a castração são os métodos cirúrgicos preferidos para controlar a reprodução no sexo masculino. Enquanto as funções sexuais terminam reversivelmente na vasectomia, elas são removidas irreversivelmente na castração. Após esses processos, são observadas alterações nos hormônios e nos parâmetros de estresse oxidativo. Neste estudo, foram investigados os efeitos das operações de vasectomia e castração nos níveis sanguíneos de hormônio folículo estimulante (FSH), hormônio luteinizante (LH), testosterona, óxido nítrico (NO) e malondialdeído (MDA) em ratos. Como resultado da análise, foi determinado que os níveis de FSH, LH, NO e MDA aumentaram ($P<0,05$) e os níveis de testosterona diminuíram ($P<0,05$) no grupo de castração em comparação com os grupos sham e vasectomia. Considerando os dados obtidos no presente estudo, quando comparadas as duas operações (vasectomia e castração) em ratos, preferidas para o controle da reprodução, acredita-se que a vasectomia é um método mais saudável por ser reversível, não afetar os níveis hormonais e não aumentar o estresse oxidativo.

Palavras-chave: castração, hormônios, estresse oxidativo, vasectomia

INTRODUCTION

Vasectomy is the process of eliminating sperm transfer by preventing the lumen continuity of the vas deferens. Castration is divided into chemical and surgical. Chemical castration is the process of injecting chemical agents intratestinally, intraepididally, and intravadally to inhibit spermatogenesis. Surgical castration is the operation of removing the testicles, which are the main production site of testosterone, to prevent

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the reproductive ability of animals. In surgical castration, sexual functions are terminated irreversibly (Bakır *et al.*, 2006, Polat *et al.*, 2018, Yang *et al.*, 2021, Ozcinar *et al.*, 2022).

Gonadotropin-releasing hormone (GnRH) neurons bind to a membrane receptor on pituitary gonadotropes via GnRH production. Thus, it plays a central regulatory role by stimulating the biosynthesis and secretion of FSH and LH (Altınbaş *et al.*, 2021). FSH stimulates the growth of Sertoli cells at the onset of puberty and accelerates spermatogenesis throughout life. Studies have shown that FSH is indispensable for spermatogenesis (Bhattacharya *et al.*, 2019, Mabonga, 2020). LH stimulates Leydig cells in the testis to synthesize and release testosterone (Mabonga, 2020).

Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS) and the defense capacity of available antioxidants, resulting in the redox paradox (Alahmar, 2019). Reactive oxygen species are the main expressions of oxidative stress in biological systems. As a result of ROS production, antioxidant defenses that lead to cell damage, apoptosis and death may be eliminated (Ruggeri *et al.*, 2020). Biomembranes and intracellular organelles are sensitive to attacks by oxidants due to the presence of unsaturated fatty acids in membrane phospholipids. MDA is one of the most important products of lipid peroxidation. MDA causes cross-linking of membrane compounds by affecting ion exchange in cell membranes, leading to negative consequences such as changes in ion permeability and enzyme activity. MDA can react with the nitrogen bases of DNA and is therefore mutagenic, genotoxic to cell cultures and carcinogenic (Eken, 2016).

NO is an important cellular signaling molecule that participates in various physiological functions in mammals, including neurotransmission, immune response vasodilation and smooth muscle relaxation. NO, a free radical, is produced by the oxidation of L-arginine (L-Arg) to L-citrulline by a family of enzymes called nitric oxide synthases (NOSs). The significant amount of NO produced helps to defend against invasive pathogens and as such is critical for the inflammatory response and the congenital immune system. However, inappropriately high NO concentrations caused by overexpression or dysregulation of inducible nitric oxide synthase (iNOS) can cause toxic effects. As a result, various diseases can be shaped, including cardiac dysfunction, diabetes, cancer, septic shock, and pain (Cinelli *et al.*, 2020).

The aim of this study was to comparatively evaluate some hormone and oxidative stress parameters in rats that had undergone castration or vasectomy operations.

MATERIALS AND METHODS

The study was conducted in the Van YYU Experimental Medicine Application and Research Center Directorate with the permission of the Van Yüzüncü Yıl University Animal Experiments Local Ethics Committee with the decision dated 28.10.2021 and numbered 2021/10-09.

Adult, pathogen-free, male Albino Wistar rats were obtained from Van Yuzuncu Yil University. Animals aged 3-4 months on average and weighing 200-250 grams were used in the study. Animals were fed *ad libitum* and kept with 12 hours of light and 12 hours of dark per day. The living areas had an average temperature of 26 °C and 60% relative humidity. Eighteen Wistar albino male rats, average 250g, were used in the study. The rats were randomly divided into 3 groups of six. To provide general anesthesia to the rats, 3mg/kg xylazine hydrochloride and 90mg/kg ketamine hydrochloride were administered intraperitoneally. Surgical procedures were carried out in a sterile environment. In addition, after the rats were placed under general anesthesia, the operation areas were shaved and sterilized.

1. Group (Control): A randomly selected 6-rat sham group was established. In rats in this group, a 2 cm-wide lower abdominal region was opened by incision and closed without any procedure.

2. Group (Vasectomy): Six randomly selected rats were planned as the vasectomy group. A vasectomy was performed on rats in this group. The 2 cm-wide lower abdominal region was opened, and the vas deferens were ligated (Contuk *et al.*, 2012) with 4-0

polypropylene non-absorbable suture. After this procedure, the region was closed with 3-0 polypropylene non-absorbable suture (Figure 1).

3. Group (Castration): The castration group included 6 randomly selected rats. Castration operation was applied to the rats in this group. The scrotum was opened and funiculus spermaticus was ligated with 4-0 polypropylene non-absorbable suture and testes were removed (Kul *et al.,* 2012). After this procedure, the region was closed with 3-0 polypropylene non-absorbable suture.

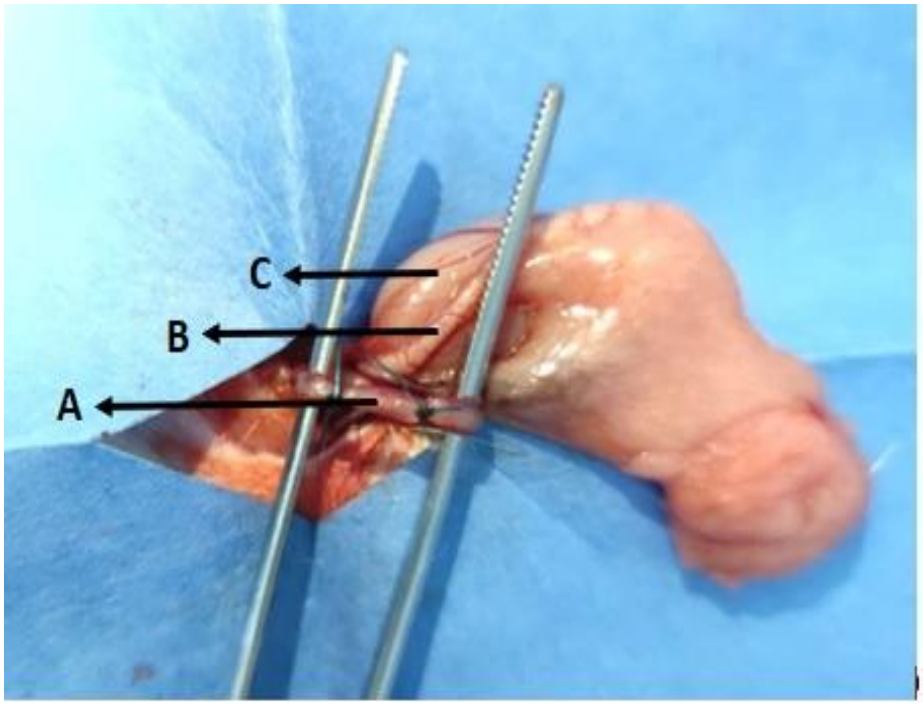

Figure 1. Vasectomy operation in rats. (A: Vas deferens, B: Epididymis, C: Testis).

After the 10th day of the surgical procedures, all rats were given general anesthesia before being sacrificed. Then, the rats were sacrificed by collecting blood from the heart. The blood samples were sent to the laboratory for oxidative stress and biochemical parameter analysis.

Plasma NO concentrations were determined colorimetrically by the chemical methods described by Miranda *et al*. (2001). Based on the Griess reaction, the sum of the nitrate and nitrite concentrations represented the amount of NO (µmol/L).

Plasma MDA concentrations were determined by a pre-established protocol (Yoshiko *et al.,* 1979). In summary, since MDA reacts with thiobarbituric acid (TBA) at low pH to produce a pinkish-red colored chromogen, the MDA concentration was determined spectrophotometrically at 532 nm using a standard curve (nmol/mL).

Blood serum samples were analyzed according to the FSH (AD3200 Ra), LH (AD1683 Ra) and testosterone (AD 1386 Ra) ELISA kit protocols of Andy Gene USA.

Data were analyzed using the SPSS Windows 20.0 statistical software package. Mean values and standard deviations were calculated for each of the evaluated indicators. One-way analysis of variance (ANOVA) was used to evaluate all parameters followed by Duncan's test. Significant results were defined as those with a value of $p<0.05$.

RESULTS

The parameters measured after the operation of rats in the castration group in the current study

showed significant changes, however the parameters measured after the operation of rats in the sham and vasectomy groups showed no statistically significant changes.

Blood serum samples were analyzed according to the FSH, LH and Testosterone ELISA kit protocols. After the surgical procedures of rats, serum LH (233.11±20.13 vs. 189.30±53.98 and 195.64±18.29 pg/mL, respectively; $p < 0.05$) and FSH (251.24±18.57 vs. 202.42±26.47 and 214.51±59.50 pg/mL, respectively; $p < 0.05$) concentrations were higher in the castration group than in the sham and vasectomy groups, while testosterone (53.27±5.02 vs. 168.01±13.32 and 181.47±12.50 pg/mL, respectively; $p < 0.05$) hormone concentrations were lower ($p < 0.05$) (Table 1).

Table 1**.** Serum mean (±SD) LH, FSH, Testosterone concentrations following surgical procedures in rats

Procedure	Parameters		
	LH (pg/mL)	FSH (pg/mL)	Testesterone (pg/mL)
Sham	189.30±53.98 [a]	202.42±26.47 [a]	168.01±13.32 [a]
Vasectomy	195.64±18.29 [a]	214.51±59.50 [a]	181.47±12.50 [a]
Castration	233.11±20.13 [b]	251.24±18.57 [b]	53.27±5.02 [b]

*The difference between groups with different (a, b) signs in the same column is significant ($p < 0.05$).

Plasma NO concentrations were determined colorimetrically by the chemical methods and plasma MDA concentrations were determined spectrophotometrically. MDA (26.56±3.02vs. 14.63±0.90 and 16.35±1.02 nmol/mL, respectively; $p < 0.05$) and NO (72.29±2.93 vs. 41.40±1.87 and 43.98±2.23 µmol/L, respectively; $p < 0.05$) concentrations measured from plasma samples were significantly higher in the castration group than in the sham and vasectomy groups ($p < 0.05$) (Table 2).

Table 2**.** Plasma mean (±SD) MDA and NO concentrations following surgical procedures in rats.

Procedure	Parameters	
	MDA (nmol/mL)	NO (µmol/L)
Sham	14.63±0.90 [a]	41.40±1.87 [a]
Vasectomy	16.35±1.02 [a]	43.98±2.23 [a]
Castration	26.56±3.02 [b]	72.29±2.93 [b]

*The difference between groups with different (a, b) signs in the same column is significant ($p < 0.05$).

DISCUSSION

Testosterone is the main androgen in males. Testosterone is a neuroactive steroid hormone that is not only related to reproduction in the body but also serves to modulate carbohydrate, protein and lipid metabolism and has an effective role in the nervous system through androgen receptors (Muthu and Seppan, 2020, Guades *et al.,* 2022). Vasectomy with surgical ligation of the vas deferens is one of the male reproductive control methods (Duru *et al.,* 2013). While no change in testosterone level is observed in vasectomy (Yang *et al.,* 2021), testosterone level decreases in castration, which is the process of removing the testicles from the scrotal sac (Turk and Ataman, 2016). Similarly, in the present study, it was observed that while testosterone levels were lower in rats that underwent castration than in rats in the sham and vasectomy groups ($p<0.05$), testosterone levels in rats that underwent vasectomy were similar to those in the sham group ($p>0.05$) and were higher than those in the castration group ($p<0.05$) (Table 1).

Pulsatile release of GnRH from the hypothalamus stimulates the release of FSH and LH. FSH plays a crucial role in the development and regulation of both the male and female reproductive systems. FSH regulates the mitotic proliferation of Sertoli cells, supports their growth and maturation, and releases androgen-binding protein, which regulates the overall process of spermatogenesis (Lizneva *et al.,* 2019). LH is part of the neurological pathway that consists of the pituitary gland,

hypothalamus, and gonads. In this pathway, LH release is stimulated by GnRH and inhibited by testosterone in males (Nedresky and Singh, 2019). FSH synergizes with testosterone and acts to stimulate all spermatogenic steps. FSH is inhibited from the pituitary by the Sertoli cell peptides inhibins (inhibin A and inhibin B) without affecting LH secretion (Dutta *et al.*, 2019). Considering this, there was no significant change in FSH and LH values ($p > 0.05$) in vasectomy-operated rats and in the sham group, while FSH and LH increased in castrated rats ($p < 0.05$) (Table 1).

Oxidative stress can play an essential role in the pathogenesis of cardiovascular diseases such as hypertension, hypertrophy, ischemic heart disease, atherosclerosis, cardiomyopathies, and congestive heart failure (Kłapcińska *et al.*, 2008). Testosterone depletion results in neuronal damage or apoptosis via oxidative stress. Oxidative damage is a condition that also causes various neurodegenerative diseases, such as Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease, cerebrovascular disorders, demyelinating diseases, and psychiatric disorders (Muthu and Seppan, 2020). Aengwanich *et al.* (2019) found that oxidative stress parameters decreased after castration in dogs. In this study, an increase in oxidative stress parameters were observed in castrated rats. Liu *et al*. (2014) found that oxidative stress parameters increase after vasectomy in rats, but in this study, no significant changes were observed in oxidative stress parameters after vasectomy in rats. In this study, different results were found from the studies of Liu *et al*. (2014) and Aengwanich *et al.* (2019) (Table 2).

While testosterone levels are not affected after vasectomy, they slowly begin to decrease after castration (Bhindi *et al.*, 2017; Chin and Ima-Nirvana, 2017). Age-related decreases in testosterone levels are a factor that increases the risk of prostate cancer. This is related to the fact that the decrease in testosterone increases oxidative stress, which leads to cancer because of DNA damage (Ide *et al.*, 2012). At the end of the study, an increase in oxidative stress parameters was observed in castrated rats, while it was not observed in vasectomized rats. It is expected that oxidative damage may occur more frequently in castrated rats, and accordingly, a decrease in the quality of life and more exposure to pathological conditions are expected to occur in rats.

CONCLUSION

Considering the results of this study, it is thought that vasectomy is healthier because it does not increase oxidative stress parameters and does not affect reproductive hormone levels in rats. This study will guide human and animal studies on the preferred vasectomy and castration operations for the control of reproduction.

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TS and VK performed the research, VK and CO analysed the data, TS, VK and CO designed the research study and wrote the paper.

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