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Investigation of Reproductive Parameters in Male Geriatric (3 years old) Rats

Volkan KOŞAL[1,*] Ömer Faruk KELEŞ[2] Ahmet Ufuk KÖMÜROĞLU[3] Yıldıray BAŞBUĞAN[4]

[1] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Artificial Insemination, 65080, Van, Türkiye*
[2] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Pathology, 65080, Van, Türkiye*
[3] *Van Yuzuncu Yil University, Health Service Vocational School of Higher Education, 65080, Van, Türkiye*
[4] *Van Yuzuncu Yil University, Faculty of Veterinary Medicine, Department of Internal Medicine, 65080, Van, Türkiye*

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ABSTRACT Many health problems are seen due to aging. One of these is problems in the reproductive system. Reproductive system problems are caused by lower urinary system symptoms, prostate diseases, low fertility, testicular dysfunction. The aim of this study was to compare reproductive parameters of geriatric (3 years old) and young (3 months old) rats. A 3-year-old rat is equivalent to an average 90-95-year-old human. For this purpose, sperm analysis, testicular and prostate histopathology, testicular oxidative stress parameters were examined in geriatric (3 years old) and young (3 months old) rats. In the analysis results, it was determined that sperm motility ratio decreased ($p<0.001$), abnormal sperm ratio increased ($p<0.001$) and sperm density decreased ($p<0.001$) in the geriatric group. Statistically, oxidative stress parameters MDA, AOPP, T-SH levels increased ($p<0.05$) and CAT level decreased ($p<0.05$) in geriatric group. Histopathologically, degeneration, necrosis and irregular alignments were observed in the tubulus seminiferous contortus in the geriatric group. Hyperplasia and dilatation of the prostate gland were detected in the geriatric group. As a result of this study, it is thought that reproductive performance in geriatric male rats is very low, and the probability of reproduction is very difficult.

Keywords: *Geriatri, Prostate, Rat, Sperm, Testis.*

ÖZ

Geriatrik (3 Yaşlı) Erkek Ratlarda Üreme Parametrelerinin Araştırılması

Yaşlanmaya bağlı olarak pek çok sağlık sorunu görülmektedir. Bu sorunlardan biri de üreme sistemindeki problemlerdir. Üreme sistemi sorunlarına alt üriner sistem semptomları, prostat hastalıkları, düşük dölverimi, testis fonksiyon bozuklukları neden olur. Bu çalışmadaki amaç geriatrik (3 yaş) ve genç (3 aylık) sıçanların üreme parametrelerinin karşılaştırılması oldu. 3 yaşındaki bir rat ortalama 90-95 yaşındaki bir insana denk gelmektedir. Bu amaçla geriatrik (3 yaş) ve genç (3 aylık) ratlarda sperm analizi, testis ve prostat histopatolojisi, testiküler oksidatif stres parametreleri incelendi. Analiz sonuçlarında geriatric grupta sperm motilite oranının düştüğü ($p<0.001$), anormal sperm oranının arttığı ($p<0.001$), sperm yoğunluğunun azaldığı ($p<0.001$) belirlendi. İstatistiksel olarak geriatric grupta oksidatif stress parametrelerinin MDA, AOPP, T-SH seviyelerinin arttığı ($p<0.05$) ve CAT seviyesinin düştüğü ($p<0.05$) tespit edildi. Histopatolojik olarak geriatrik grupta tubulus seminiferous contortuslarda dejenerasyon, nekroz ve düzensiz dizilimler gözlendi. Geriatik grupta prostat bezinde hiperplazi ve dilatasyon tespit edildi. Bu çalışma verileri sonucunda geriatrik erkek ratlarda üreme performansının çok düşük olduğu ve üreme olasılığının çok zor olduğu düşünülmektedir.

Anahtar Kelimeler: *Geriatri, Prostat, Sıçan, Sperm, Testis.*

INTRODUCTION

Aging is a natural process observed in all living species. The age of 65 and over is accepted as geriatric throughout the world. Many health problems are encountered in this process. It can be classified as digestive, respiratory, circulatory, excretory, nervous, musculoskeletal, reproductive systems (Elsawy and Higgins 2011, Kammerlander et al. 2010; Thakur et al. 2013). Reproductive system problems are caused by lower urinary system symptoms, prostate diseases, low fertility and testicular dysfunction (Corona et al. 2010; Auerbach et al. 2012; Donna et al. 2015).

Many changes occur in the testis due to aging; reduction in volume, dysfunction in Sertoli cells, decrease in the number of germ cells, degeneration of Leydig cells, decrease in testosterone production etc. Depending on these changes, decrease in sperm count, increase in

✉ *Corresponding author: volkan_kosal@hotmail.com

abnormal sperm ratio, increase in dead-live sperm ratio, decrease in motility ratio, and increase in sperm DNA damage can be observed (Hu et al. 2013; Lindor 2014; Francisco et al. 2015; Mattigk et al. 2020).

Changes in androgen metabolism and regulation of apoptosis cause enlargement of the prostate gland. This problem, called benign prostatic hyperplasia, is thought to be caused by the more active form of testosterone, dihydrotestosterone (DHT). It is a common problem in older men (Rosette et al. 2001; McVary 2006; Kramer et al. 2006; Raymond et al. 2009).

Reactive oxygen species and free radicals increase with aging. Free radicals have a direct effect on cell growth and development, and from these direct effects on cell life; imbalance in redox homeostasis occurs, the antioxidant enzyme mechanism cannot function adequately, and radical formation increases. ROS attack DNA, protein, lipids and molecules in all structures. It causes cell membrane damage with DNA molecule oxidation, genetic messenger DNA damage, cell division arrest, uncontrolled growth, malignancy, lipid peroxidation (Sabuncuoğlu and Özgüneş 2011; Öğüt and Atay 2012; Tabakoğlu and Durgut 2013; Özcan et al. 2015).

The aim of this study is to compare the changes in reproductive parameters, testicular oxidative stress and testicular histopathology in geriatric and young rats.

MATERIAL AND METHODS

The study was undertaken under agreement no. 2021/12-06 of Van Yuzuncu Yil University Animal Experiments Local Ethics Committee, dated 23/12/2021.

Animals

Adult, pathogen-free, male Albino Wistar rats were obtained from Van Yuzuncu Yil University. 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.

Groups

We used 16 Albino Wistar rats.

Group 1 (n:8): 3-4 months old animals with an average weight of 150-200 gr (Figure 1 B). Group 2 (n:8): 36 months old animals with an average weight of 400-450 gr (Figure 1 A).

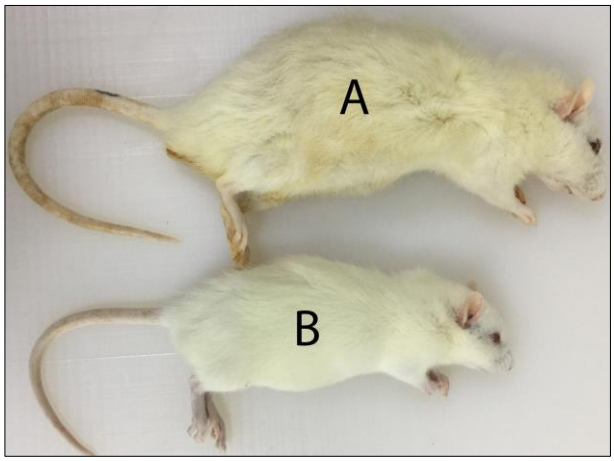

Figure 1: Macroscopic view of young and geriatric rat. A: Macroscopic view of geriatric group rats. B: Macroscopic view of young group rats.

Sperm Examination

Motility examination: The sperm sample was obtained by epididymis puncture immediately after sacrifice and was placed on a glass slide on the heating table set to 38 °C. The coverglass was closed at an angle of 45° and motility (in %) detected by microscopy at 40x magnification (Hafez and Hafez 2016).

Density analysis: After epididymal puncture, 0.1 ml of sperm sample was added to Eppendorf tubes with 0.5 ml Hayem solution (Norateks, Germany). Sperm count per ml was calculated on a Thoma cell counting chamber (Hafez and Hafez 2016).

Abnormal sperm ratio: The sperm obtained by epididymis puncture was transferred to Eppendorf tubes with 0.5 ml Hancock solution (Norateks, Germany). At least 400 sperm samples were examined at 40x magnification to determine the ratio (Hafez and Hafez 2016).

Histopathological Examination

At the end of the experiment, necropsies of the rats were performed, testis and prostate tissue samples were taken, and the observed macroscopic changes were recorded. After the tissue pieces were fixed in 10% buffered formaldehyde solution, routine tissue follow-up was performed and embedded in paraffin blocks and 4 µm sections were taken with a microtome. They were stained with hematoxyleneosin (H&E) and examined under a light microscope, and morphological findings were photographed and evaluated.

MDA, AOPP, T-SH, CAT

Total sulphydryl content (protein and non-protein Thiols) was measured based on the method of Sedlak and Lindsay (1968). Advanced oxidation protein products, AOPP was determined via the method described by Witko-Sarsat et al. (1996). Testis tissue MDA level was measured by the method identified by Ohkava et al. (1979) and MDA level was presented as mmol/gr tissue. CAT activity was spectrophotometrically analyzed at 240 nm according to the Lartillot and Kedziora (1988) method.

Statistical Analysis

SPSS v.20 (Chicago, IL, USA) package program was used for statistical analysis. All data were expressed as mean ± standard deviation. Statistical analyzes of the groups were analyzed statistically using the One-way ANOVA followed by post hoc multiple comparisons (Tukey's test) for comparative analysis between the groups. $P<0.05$ was regarded as statistically significant.

RESULTS

Sperm Examination

Sperm motility and density were significantly decreased in geriatric group, and abnormal spermatozoa rate was significantly increased in geriatric group respectively ($p<0.001$).

Oxidative Stress

Testicular tissue MDA, AOPP and T-SH levels in geriatric rats were significantly higher when compared to testicular tissue of young rats ($p=0.023$, $p=0.000$, $p=0.000$, respectively). Testicular tissue CAT activity was found to be significantly lower in geriatric rats compared to young rats ($p=0.019$).

Histopathology

Testis

Normal histological structure of testicles was observed in the young group (Figure 2 A). In the geriatric group, tubulus seminiferus contortus were observed in an irregular manner, which lost their normal structure widely. It was determined that there was a significant decrease in tubules and spermatogenesis, and primary spermatogonium cells were hyperchromatic-pycnotic. In addition, degenerative-necrotic changes in spermatogonia and lysis were observed in some tubules (Figure 2 B, C). Enlargement of the interstitium was observed as a result of proliferation (fibromuscular hyperplasia) in smooth muscle cells in the intertubular region (Figure 2 D).

Prostate

While the prostates of the young group rats were macroscopically normal (Figure 3 A), the prostates of the geriatric group rats were found to be much larger than normal (Figure 3 B). Normal histological structure was observed in the prostates of young rats (Figure 3 C). In the geriatric group, dilatation of the prostate glands and hyperplasia in the form of papillary extensions towards the lumen were detected in some parts (Figure 3 D).

Table 1: Sperm motiliy, density and abnormal sperm rate of geriatric and young rats.

	Young Group	Geriatric Group	p
Motility (%)	82.5±4.62	26.25±9.16	<0.001
Density (x10^9 /ml)	2.34±0.21	1.06±0.16	<0.001
Abnormal sperm (%)	13.37±3.2	41.12±7.58	<0.001

Table 2: MDA, AOPP and T-SH levels and CAT activity in testicular tissue of geriatric and young rats.

	Young Group	Geriatric Group	p
MDA (nmol/gr tissue)	0.60±0.06	0.71±0.07*	0.023
AOPP (mmol/gr tissue)	16.82±1.44	21.07±1.20*	0.000
T-SH (mmol/gr tissue)	0.45±0.02	0.54±0.03*	0.000
CAT (U/L)	449.29±64.74	358.45±41.91*	0.019

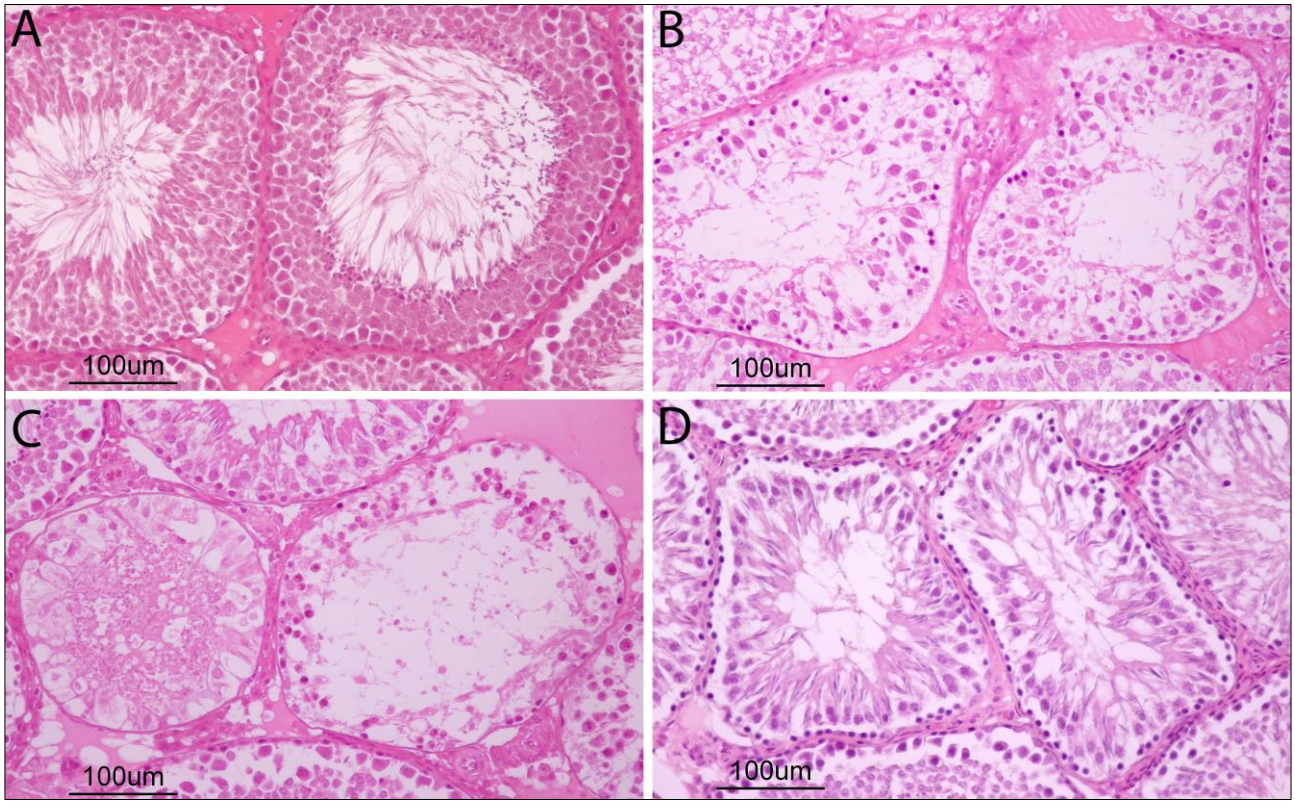

Figure 2: Testis histopatology of geriatric and young rats. A: Young Group: Normal histological appearance of testis. B-C-D: Geriatric Group: Reduction in tubules and spermatogenesis, degenerative-necrotic changes and lysis. Fibromuscular hyperplasia in the intertubular region. H.E., Bar; 100.

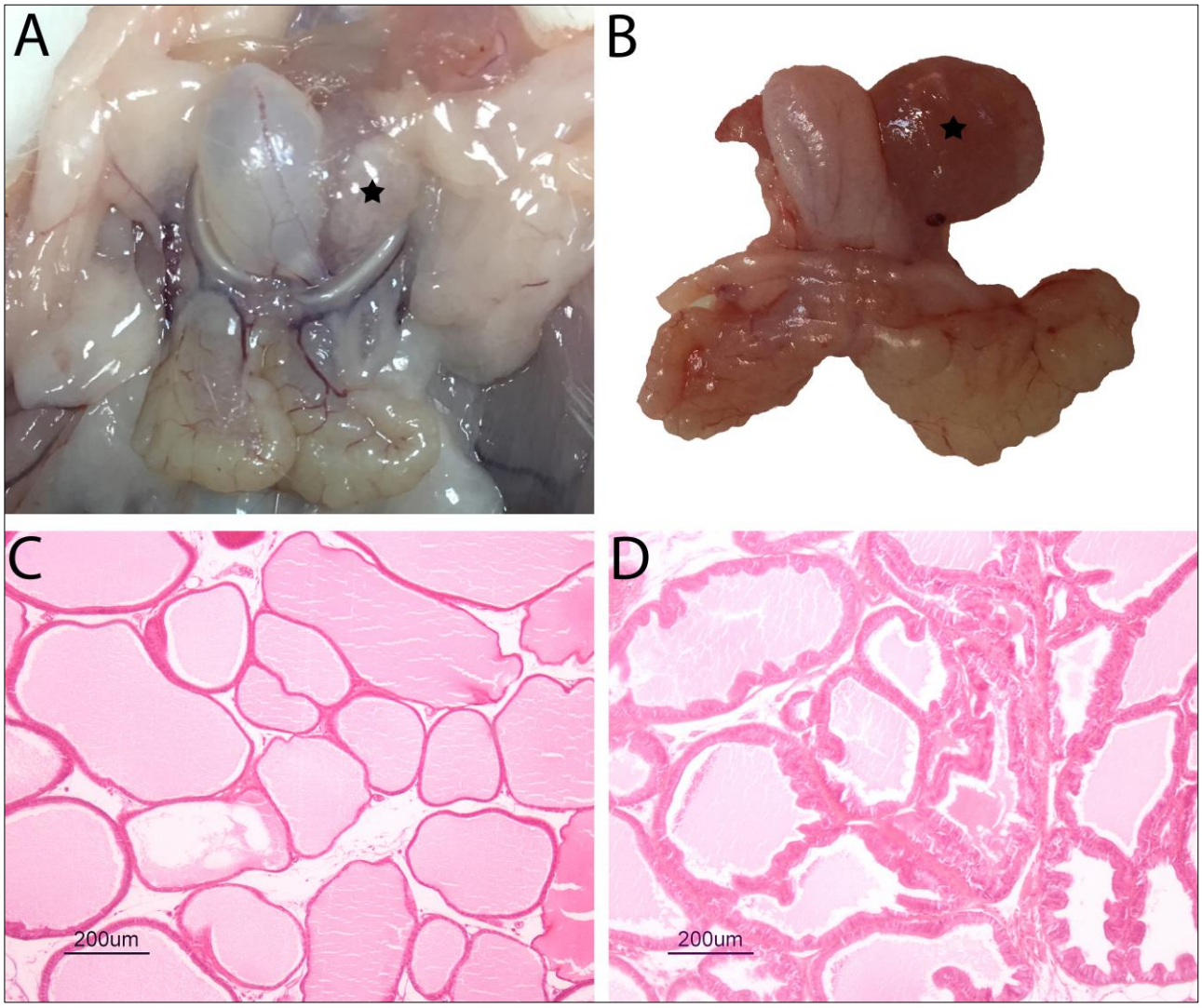

Figure 3: Prostate macroscopy and histopatology of geriatric and young rats. Macroscopy of prostates of young group (A) and Geriatric group (B) rats. C: Normal histological appearance of prostates of young rats, H.E. Bar; 200. D: Prostate hyperplasia of geriatric rats, H.E., Bar; 200.

DISCUSSION AND CONCLUSION

Aging and dying are natural and irreversible processes in all living things. Aging is often associated with a range of diseases and disorders in a healthy lifestyle. One of them is reproductive system problems.

The most important reproductive system problems are fertility problems. Reproductive problems are associated with motility, abnormal sperm and density. In this study, it was determined that motility, abnormal sperm ratio and density were negatively affected from sperm parameters ($p<0.001$). Many researchers stated that they detected a decrease in motility, an increase in the number of abnormal sperm, a decrease in density and an increase in sperm DNA damage (Berry et al. 1989; Haidl et al. 1996; Lucio et al. 2013). The increase in sperm DNA damage is caused by the increase in microdelations in the Y chromosome, telomere length, oxidative stress, gene mutations, DNA methylations and decrease in DNA repair mechanism capacity due to aging (Dong et al. 2022).

Reasons for reproductive system problems; dysfunction in Leydig cells, impaired testicular perfusion, decreased steroid synthesis, hypothalamus and pituitary dysfunction, testicular tubular membrane fibrosis, diverticula formation, decreased number of type A spermatagonia, increased number of atypical and giant spermatogonia, desquamation of immature germ cells, malformed spermatid, degeneration in sertoli cells, increased ROS level etc. (Berry et al. 1989; Gallardo et al. 1996; Haidl et al. 1996; Lucio et al. 2013; Santi et al. 2017). These problems are observed due to aging. In this study, testicular histopathology showed irregular alignment in the tubulus seminiferus contours, decreased spermatogenesis, hyperchromatic and pycnotic primary spermatogonia, degeneration and necrosis in the tubules.

Increase in oxidative stress parameters due to aging Robaire and Hales (2003), Sakamoto (2009), Ingles et al. (2014), Demir et al. (2014) stated by the researchers. In this study, the results of the histopathological examination of the testicles and the oxidative stress parameters (MDA, AOPP, T-SH, CAT) examined in the testicular tissue in the geriatric group are consistent with the negativities in the

sperm parameters. With advancing age, the level of semen ROS increases. ROS oxidizes guanine to 8-hydroxy-20-deoxyguanosine (8OHdG). 80 HdG is one of the most important markers in the capacity of spermatozoa to withstand ROS. The increase in ROS (MDA, SOD, AOPP) levels and 80 HdG oxidation cause lipid peroxidation, DNA damage, enzyme inactivation and protein oxidation, leading to infertility problems (Castleton et al. 2022).

In studies on geriatric male rats, many researchers reported that the prostate gland became hyperplasia after 24 months because of impaired testosterone metabolism. In this study, in which 36-month-old male rats were used, BPH formation due to aging was determined histopathologically. Studies have shown that the amounts of citric acid, spermine, spermidine putrescine, testosterone, protein, fructose, and PGE in the seminal plasma are decreased. These adverse events are directly related to BPH (Rui et al. 1986; Sloter et al. 2006; Smithson et al. 2019; Ross et al. 2019; Sharma et al. 2020).

In studies using geriatric rats, some researchers have stated that reproduction and sexual behaviors can continue in a healthy way. The age of the rats used in these studies was limited to 12-24 months (Berry et al. 1989; Lucio et al. 2013; Muselin et al. 2019; Felipe et al. 2019). When the rats used in this study are 36 months old and the results obtained are compared, it is observed that the probability of healthy reproduction is very low.

As a result of this study, it was determined that there were degenerations in testicular tissue, increase in oxidative stress and BPH due to aging. Due to these reasons, a decrease in sperm motility and density, and an increase in abnormal spermatozoa were detected. For these reasons, it is thought that geriatric males have very low reproductive performance, and the possibility of reproduction is very difficult.

CONFLICTS OF INTEREST

The authors report no conflicts of interest for this study.

AUTHOR CONTRIBUTIONS

Idea / Concept: VK, YB
Supervision / Consultancy: YB
Data Collection and / or Processing: ÖFK, AUK
Analysis and / or Interpretation: AUK, YB
Writing the Article: VK
Critical Review: YB, ÖFK, AUK

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