



The Effect of Orally Administered Collagen on Testosterone Hormone and Sperm Count in Streptozotocin-Applied Rats

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ABSTRACT

Diabetes mellitus is a common metabolic disorder characterized by chronic hyperglycemia resulting from impaired insulin secretion or action. Collagen, the most abundant structural protein in the body, plays a critical role in tissues such as skin, bone, cartilage, blood vessels, and testis. This study aimed to investigate the effects of oral collagen supplementation on serum testosterone levels and sperm count in diabetic rats. A total of 32 male Wistar albino rats were randomly assigned into four groups: Control, Diabetes mellitus, Diabetes mellitus+Collagen, and Collagen. Diabetes mellitus was induced via a single intraperitoneal injection of streptozotocin (55 mg/kg), while collagen peptide was administered daily by gastric gavage at a dose of 600 mg/kg for 28 days. Serum testosterone levels were measured using Enzyme-Linked Immunosorbent Assay (ELISA). The results demonstrated a significant decrease in testosterone ($p<0.05$) and sperm count ($p<0.001$) in the Diabetes mellitus group, whereas the Diabetes mellitus+Collagen group exhibited partial improvement in both parameters. In the Collagen-only group, testosterone levels and sperm count were comparable to the Control group, indicating a limited effect of collagen under normal physiological conditions. These findings suggest that collagen may partially mitigate diabetes-induced testicular dysfunction by supporting the structural integrity of testicular tissue and promoting spermatogenesis. Overall, the results indicate that collagen supplementation could support testicular function under diabetic conditions, but further studies with larger sample sizes and longer durations are warranted to better elucidate its therapeutic potential.

Keywords: Collagen, Diabetes mellitus, Rat, Sperm, Testosterone.

ÖZ

Streptozotocin Uygulanan Ratlarda Oral Yolla Verilen Kolajenin Testosteron Hormonu ve Sperm Sayısı Üzerindeki Etkisi

Diyabetes mellitus, insülin salgısının veya etkisinin bozulmasından kaynaklanan kronik hiperglisemi ile karakterize yaygın bir metabolik bozukluktur. Vücutta en bol bulunan yapısal protein olan kolajen, deri, kemik, kıkırdak, kan damarları ve testis gibi dokularda kritik bir rol oynar. Bu çalışma, oral kolajen takviyesinin diyabetik sıçanlarda serum testosteron düzeyleri ve sperm sayısı üzerindeki etkilerini araştırmayı amaçlamıştır. Toplam 32 erkek Wistar albino sıçan, rastgele dört gruba ayrılmıştır: Kontrol, Diyabetes mellitus, Diyabetes mellitus+Kolajen ve Kolajen. Diyabetes mellitus, tek bir intraperitoneal streptozotocin enjeksiyonu (55 mg/kg) ile indüklenmiş, kolajen peptidi ise 28 gün boyunca günlük olarak gastrik gavaj yoluyla 600 mg/kg dozunda uygulanmıştır. Serum testosteron düzeyleri ELISA yöntemi ile ölçülmüştür. Sonuçlar, Diyabetes mellitus grubunda testosteron ($p<0.05$) ve sperm sayısında ($p<0.001$) anlamlı bir azalma olduğunu göstermiştir; buna karşın Diyabetes mellitus+Kolajen grubunda her iki parametrede kısmi bir iyileşme gözlenmiştir. Sadece Kolajen verilen grupta, testosteron düzeyleri ve sperm sayısı Kontrol grubu ile karşılaştırılabilir düzeyde bulunmuş ve normal fizyolojik koşullar altında kolajenin sınırlı bir etkisi olduğu görülmüştür. Bu bulgular, kolajenin testis dokusunun yapısal bütünlüğünü destekleyerek ve spermatogenezi teşvik ederek diyabete bağlı testiküler disfonksiyonu kısmen azaltabileceğini göstermektedir. Genel olarak, sonuçlar kolajen takviyesinin diyabetik koşullar altında testis fonksiyonunu destekleyebileceğini göstermektedir; ancak terapötik potansiyelini daha iyi anlamak için daha büyük örneklem büyüklükleri ve daha uzun süreli çalışmalar gerekmektedir.

Anahtar Kelimeler: Kolajen, Rat, Sperm, Şeker hastalığı, Testosteron.



INTRODUCTION

Diabetes mellitus represents a significant metabolic disorder that affects multiple organ systems and exerts considerable negative impacts on male reproductive health (Jena et al. 2024; Jin et al. 2025). Its global prevalence continues to rise, with type 2 diabetes comprising the majority of diagnosed cases (Lovic et al. 2020; Lotti and Maggi 2023). Both clinical observations and experimental studies have indicated that this metabolic condition induces extensive and multifactorial damage within the male reproductive system (Huang et al. 2024; Chen et al. 2025). While the precise mechanisms underlying diabetes-related male reproductive dysfunction remain incompletely understood, current research identifies testicular impairment, disruptions in spermatogenesis, and structural changes in the testicular microenvironment as central pathological features (Faheem et al. 2025).

Diabetes disrupts the structure of the basement membrane through advanced glycation end-products and oxidative stress, leading to membrane thickening at the microvascular level and impairing ECM stability (Tsilibary 2003). In the testis, this process has been associated with increased expression of type IV collagen and laminin $\alpha 5$, with ECM accumulation weakening basement membrane integrity and adversely affecting spermatogenesis (Khordad et al. 2020). Furthermore, the observed elevation of type I collagen levels in diabetes has been linked to impaired seminiferous tubule morphology, germ cell loss, and reduced spermatogenesis, supporting the critical role of ECM balance in maintaining testicular function (Bondarenko et al. 2012).

Diabetes has been reported to diminish testicular antioxidant defenses and trigger germ cell apoptosis, resulting in decreased testosterone production. Reduced testosterone levels subsequently impair epididymal function and overall reproductive competence (Kiani et al. 2025). Experimental studies in diabetic rat models further demonstrate significant alterations in sexual behavior, accompanied by lowered serum concentrations of testosterone, Follicle Stimulating Hormone (FSH), and Luteinizing Hormone (LH) (Abdel-Wahab et al. 2024).

Collagen is the most plentiful structural protein in the body and is essential for the integrity of various tissues, such as skin, bones, cartilage, blood vessels, and testes (Shoulders and Raines 2009). It is particularly abundant in the basal membrane of seminiferous tubules and the interstitial regions, where it helps maintain the microenvironment required for spermatogenesis, ensures the stability of the blood–testis barrier, and supports germ cell differentiation (Cheng et al. 2010). In diabetic conditions, disruptions in collagen balance within the basal membrane, along with excessive connective tissue accumulation, have been linked to reduced sperm production (Xu et al. 2025).

This study aims to evaluate the effects of oral collagen supplementation on testosterone levels and sperm count in rats modeled with streptozotocin-induced diabetes. The limited number of studies examining the effects of collagen on male reproductive function in diabetic patients constitutes the unique value of this study. In this context, the study aims to demonstrate the potential role of collagen in reducing diabetes-related fertility disorders through a holistic approach.

MATERIAL AND METHODS

The experimental animals utilized in this study were sourced from the Experimental Research Application and Research Center of Van Yuzuncu Yil University. A total of 32 adult male Wistar albino rats, aged 8–12 weeks and with comparable average body weights, were included in the study. Throughout the experimental period, animals were kept under standard laboratory conditions including 23 ± 1 °C temperature, 50–60% relative humidity, and a 12-h light/12-h dark cycle. Sterile drinking water and standard pellet chow were provided ad libitum. Rats were housed individually in polycarbonate cages with wood shavings as bedding, and cages were cleaned twice weekly. All animal experiments were carried out following prior approval from the Local Ethics Committee on Animal Experiments at Van Yuzuncu Yil University (Date: 28.08.2025 Approval No: 2025/09-02) and adhered to all applicable ethical standards and guidelines.

Induction of the Diabetes Model

The experimental diabetes model was created using streptozotocin (STZ). Diabetes was induced by a single intraperitoneal injection of STZ at 55 mg/kg, after it was dissolved in 0.1 M citrate buffer (pH 4.5). Seventy-two hours after the injection, blood samples were obtained from the tail vein, and glucose levels were measured using a glucometer. Rats exhibiting blood glucose concentrations above 200 mg/dL were considered diabetic (Kumar et al. 2016; Maranduca and Serban 2022).

Formation of Experimental Groups

A total of 32 male Wistar albino rats were randomly allocated into four experimental groups as follows:

Control group: Rats in this group did not receive any treatment.

Diabetes mellitus group: Rats in which diabetes was induced via streptozotocin but received no subsequent intervention (Kumar et al. 2016; Maranduca and Serban 2022).

Diabetes mellitus+Collagen group: Diabetic rats received oral administration of collagen peptides at 600 mg/kg via gastric gavage for 28 consecutive days (Kumar et al. 2016; Maranduca and Serban, 2022; Vijayan et al. 2022). Collagen group: Healthy rats administered collagen peptides at 600 mg/kg orally via gastric gavage for 28 days (Vijayan et al. 2022).

Measurement of Testosterone Levels

Serum testosterone concentrations were measured using a commercial ELISA kit (Cat. No: ELK10883). The kit has a sensitivity of 0.64 ng/mL and a measurement range of 1.56–100 ng/mL.

Collection of Sperm from the Cauda Epididymis and Assessment of Sperm Count

Twenty-four hours after the completion of the experimental period, one testis from each rat was surgically excised under anesthesia. Sperm samples were obtained from the cauda epididymis according to the procedure of a previous study (Sönmez et al. 2005). In brief, the epididymides were finely minced with anatomical scissors in 2 mL of physiological saline within a Petri dish and incubated at room temperature for 15 minutes to allow complete sperm release.

Sperm concentration was measured using a slightly adapted version of the method described by Sönmez et al. (2005), in which an Eppendorf tube was used instead of the conventional hemocytometer. Ten microliters of the

epididymal suspension in physiological saline were added to 990 μL of eosin solution in an Eppendorf tube and vortexed for 15 seconds. About 10 μL of the diluted sample was placed into the counting chambers of a Thoma slide and allowed to settle for 5 minutes. Sperm cells were later analyzed and counted using a phase-contrast microscope at 400 \times magnification.

Statistical Analysis

Statistical analyses related to testosterone were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Data are presented as mean \pm standard deviation. Differences among groups were evaluated using one-way ANOVA, followed by Duncan's multiple range test for post hoc comparisons. A p -value \leq 0.05 was considered statistically significant. Differences between groups regarding sperm parameters were evaluated using one-way analysis of variance (ANOVA), and Tukey's HSD post hoc test was employed to determine significant differences.

Table 1: The effect of collagen on testosterone hormone and sperm concentration in streptozotocin-administered rats.

	Control (n=8)	Diabetes mellitus (n=8)	Diabetes mellitus+Collagen (n=8)	Collagen (n=8)	p value
Testosterone (pg/ml)	39.25 \pm 3.22 ^b	33.40 \pm 1.97 ^c	48.33 \pm 2.19 ^a	35.40 \pm 1.97 ^c	(p <0.05)
Sperm Density ($\times 10^6$)	70.37 \pm 1.59 ^b	52.62 \pm 1.06 ^c	79.37 \pm 1.40 ^a	54.25 \pm 1.03 ^c	(p <0.001)

a, b, c: Statistical differences between groups indicated with different letters in the same row are significant.

DISCUSSION AND CONCLUSION

This research examined the impact of orally administered collagen on testosterone levels and sperm count in rats with streptozotocin-induced diabetes. The findings indicate that diabetes adversely affects the male reproductive system, mainly evidenced by reduced testosterone and impaired sperm production.

In the current study, no statistically significant difference was detected between the testosterone levels of the diabetes group and those of the group receiving collagen supplementation alone. Although this result may seem unexpected at first glance, it is consistent with previous findings. In healthy individuals, testicular tissue architecture, Leydig cell function, and vascular integrity are maintained under normal physiological conditions (França et al. 2005; Skinner 2005). Consequently, exogenous collagen is not expected to substantially enhance testosterone synthesis. Collagen, as a principal component of the extracellular matrix, provides structural support to the seminiferous tubules (Hadley and Dym 1987). Furthermore, collagen within the extracellular matrix of spermatogonial stem cells contributes to a three-dimensional scaffold that preserves tubular morphology and mechanical stability (Kahsai et al. 1997). Collagen has also been reported to participate in the formation of intercellular tight junctions, in addition to its structural role (Siu et al. 2003). Therefore, the finding that testosterone levels in the collagen-only group remained comparable to the control group and did not differ significantly from the diabetes group is consistent with these established roles.

The present study demonstrated significant reductions in both sperm count and serum testosterone levels in the

RESULTS

Serum testosterone concentrations in the diabetes mellitus group were significantly reduced compared to the control and diabetes mellitus+collagen groups (p <0.05). Rats in the diabetes mellitus+collagen group displayed markedly higher serum testosterone levels than those in all other groups (p <0.05). No significant difference was observed between the collagen group and the diabetes mellitus group regarding serum testosterone levels (p >0.05).

Similarly, sperm density in the diabetes mellitus group was significantly lower than that in the control and diabetes mellitus+collagen groups (p <0.001). In contrast, sperm density in the diabetes mellitus+collagen group was significantly higher than in all other groups (p <0.001), whereas no significant difference was detected between the collagen and diabetes mellitus groups (p >0.001).

diabetic group, consistent with previous literature reports (AlTamimi et al. 2021; Imani et al. 2021; Khalil et al. 2021; Andlib et al. 2023; Hasan et al. 2023). It is widely recognized that diabetes mellitus, particularly type 2 diabetes, impairs sperm production through multiple interconnected mechanisms. The hormonal imbalances negatively affect critical stages of spermatogenesis, particularly the primary meiotic division, thereby compromising Sertoli and Leydig cell function. In addition, reductions in Sertoli cell numbers and insufficient lactate production limit the energy supply essential for germ cell survival, ultimately promoting increased germ cell apoptosis (Andlib et al. 2023; Gregorič et al. 2025).

In the present study, reductions in both sperm count and testosterone levels were observed solely in the group receiving collagen supplementation when compared to the control group, a result that aligns with previous findings (Hasan et al. 2023).

The observed increase in sperm count in the diabetes+collagen group compared with the diabetes group is a significant finding. This suggests that collagen may help maintain the integrity of the basal membrane in the testes, thereby mitigating structural disruptions within the seminiferous tubules. The extracellular matrix is known to play a regulatory role in spermatogenesis (Siu and Cheng 2008). Nevertheless, the inability of collagen supplementation to fully restore sperm count in diabetic rats is not unexpected, as pathological processes induced by diabetes, including oxidative stress and vascular damage, cannot be entirely reversed through extracellular matrix support alone. Recent evidence indicates that diabetes-associated testicular injury is multifactorial and is accompanied by substantial impairment of antioxidant defense systems. These findings underscore the importance of therapeutic strategies that simultaneously

target multiple mechanisms rather than relying on single-target interventions (Xu et al. 2023; Soetan et al. 2024; Kiani et al. 2025). The relatively small number of animals per group, inter-individual biological variability, and the inherent fluctuations in hormonal parameters and the spermatogenesis process may have limited the detection of statistically significant differences. In this context, the lack of a difference in testosterone levels between the diabetes group and the collagen-only group represents a physiologically normal finding, which can be attributed both to the limited effect of collagen on hormone production in healthy rats and to methodological constraints.

Study findings suggest that collagen may act primarily by maintaining testicular tissue integrity and supporting spermatogenesis, rather than directly increasing hormone production. In conclusion, this study provides novel findings that contribute to the existing knowledge gap regarding the effects of oral collagen administration on testosterone levels and sperm count in streptozotocin-induced diabetic rats and suggests that collagen may serve as a potential adjunctive agent in the treatment of diabetes-associated reproductive dysfunction. The lack of histopathological evaluation, lack of oxidative stress marker measurements, and inability to assess collagen bioavailability represent significant methodological limitations. Therefore, future studies investigating tissue-level changes through histological analysis, assessing oxidative stress parameters, and incorporating comparative dose-response designs are recommended.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea/Concept: SB, AUK

Supervision/Consultancy: SB

Data Collection and/or Processing: SB

Analysis and/or Interpretation: SB, AUK

Writing the Article: SB

Critical Review: SB, AUK

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