Kocatepe Veterinary Journal

https://doi.org/10.30607/kvj.1836561

Research Article

The Protective Effect of Coenzyme Q10 on Serum Anti-Müllerian Hormone Levels in Rats Treated with Cyclophosphamide

Siklofosfamid ile Tedavi Edilen Ratlarda Serum Anti-Müllerian Hormon Düzeyleri Üzerinde Koenzim Q10'un Koruyucu Etkisi

Mehmet YILDIZ[1]

[1]Department of Obstetrics and Gynecology, Faculty of Veterinary Medicine, Van Yüzüncü Yıl University, Van, Türkiye

Corresponding Author:
Mehmet YILDIZ
*mehmetyildiz@yyu.edu.tr

ORCID:
MY:0000-0001-5523-7433

Received: 05.12.2025
Accepted: 27.02.2026
Published: 15.03.2026

Citation:

Yıldız M. The Protective Effect of Coenzyme Q10 on Serum Anti-Müllerian Hormone Levels in Rats Treated with Cyclophosphamide. Kocatepe Veterinary Journal (2026) 19(1): 74-80

Abstract

Anti-Müllerian hormone (AMH) is widely used in veterinary medicine to assess reproductive health. Cyclophosphamide (CP) is a chemotherapeutic and immunosuppressive drug that has been reported to reduce AMH levels and cause damage to ovarian reserves, although findings vary depending on study design. Coenzyme Q10 (CoQ10), known for its antioxidant properties, has been suggested to have a protective role against oxidative stress-related ovarian damage. This study was conducted to examine how CP affects serum AMH levels in rats and whether CoQ10 supplementation could help reduce any negative impact. A total of 35 Wistar Albino rats were used in the study. They were divided into five groups: control, sham, CP, CoQ10, and CP+CoQ10. All substances were administered by oral gavage. CP was dissolved in distilled water and administered at a dose of 6 mg/kg/day, while CoQ10 was dissolved in olive oil and given at a dose of 2.8 mg/kg/day. The treatments lasted for four weeks. Results showed that CP alone did not significantly change AMH levels ($p>0.05$). In contrast, CoQ10 alone led to a significant increase in serum AMH levels ($p<0.05$). However, when CP and CoQ10 were given together, no significant difference in AMH levels was observed. This may suggest that CP interferes with or masks the effect of CoQ10 on the ovaries. These findings may contribute to a better understanding of the interaction between CP and CoQ10, especially in terms of dosage, duration and timing of treatment, in future studies.

Keywords: AMH, Cyclophosphamide, Coenzyme Q10, Rat

Öz

Anti-Müllerian hormon (AMH), veteriner hekimlikte reprodüktif sağlığını değerlendirmek için yaygın olarak kullanılmaktadır. Siklofosfamid (CP), AMH düzeylerini düşürdüğü ve ovaryum rezervlerine zarar verdiği bildirilen kemoterapötik ve immünosüpresif bir ilaçtır. Ancak bulgular çalışma tasarımına bağlı olarak değişiklik göstermektedir. Antioksidan özelliğiyle bilinen Koenzim Q10'un (CoQ10), oksidatif strese bağlı ovaryum hasarına karşı koruyucu bir rolü olduğu öne sürülmektedir. Bu çalışma, CP'nin sıçanlarda serum AMH düzeylerini nasıl etkilediğini ve CoQ10 takviyesinin herhangi bir olumsuz etkiyi azaltmaya yardımcı olup olamayacağını incelemek amacıyla yürütülmüştür. Çalışmada toplam 35 Wistar Albino sıçanı kullanılmıştır. Sıçanlar beş gruba ayrılmıştır: kontrol, sham, CP, CoQ10 ve CP+CoQ10. Tüm maddeler oral gavaj yoluyla uygulanmıştır. CP; distile suda çözülerek 6 mg/kg/gün dozunda uygulanırken, CoQ10 zeytinyağında çözülerek 2,8 mg/kg/gün dozunda uygulandı. Tedaviler dört hafta sürdü. Elde edilen sonuçlarda, CP'nin tek başına AMH seviyelerinde anlamlı bir değişiklik oluşturmadığı görüldü ($p>0,05$). Buna karşılık, CoQ10 tek başına serum AMH seviyelerinde anlamlı bir artışa yol açtı ($p<0,05$). Ancak CP ve CoQ10 birlikte uygulandığında, AMH seviyelerinde anlamlı bir fark gözlenmedi. Bu durum, CP'nin CoQ10'un ovaryum üzerindeki etkisini engellediğini veya maskelediğini düşündürdü. Bu bulgular, özellikle dozaj, tedavi süresi ve zamanlaması açısından, CP ve CoQ10 arasındaki etkileşimin gelecekteki çalışmalarda daha iyi anlaşılmasına katkıda bulunabilir.

Anahtar Kelimeler: AMH, Koenzim Q10, Sıçan, Siklofosfamid

Introduction

Anti-Müllerian hormone (AMH) is widely used in veterinary medicine to assess female animals reproductive health (Türk, 2016; Yildiz et al., 2025). Studies report that the source of AMH is healthy, growing ovarian follicles. AMH is not produced in atretic follicles. Therefore, an estimate of the number of ovarian follicles can be made by assessing AMH concentrations (La Marca & Volpe, 2006). In animals, AMH is also produced by granulosa cells during early follicle development, particularly in preantral and small antral follicles. Because AMH levels reflect the number of growing follicles, they are frequently used to estimate ovarian reserve in research and clinical practice (Weenen et al., 2004; La Marca & Volpe, 2006).

Cyclophosphamide (CP) is a chemotherapy drug used to treat cancer in humans and animals (Emadi et al., 2009; Gephart et al., 2025). However, CP can damage many healthy cells, including the ovaries. Studies in mice have shown that CP reduces ovarian follicles, which can lead to permanent damage to fertility (Jeelani et al. 2017). While some previous studies have found that CP reduces AMH levels, the results vary depending on the methods used (Türk, 2016; Zan & Duran, 2020).

Coenzyme Q10 (CoQ10) has antioxidant properties in cellular metabolism, regenerating other antioxidants due to its beta-like effect and scavenging free radicals. It also prevents the oxidation of lipids, proteins, and DNA (Villalba & Navas, 2000). Research suggests that CoQ10 may reduce oxidative damage, promote better follicle growth, and improve egg cell health (Ben-Meir et al., 2015; Rodríguez-Varela & Labartai 2021). While some studies report that CoQ10 supplementation increases ovarian reserve, egg quality, and ovulation rate (Bentov et al., 2014; Ben-Meir et al., 2015; Özcan et al., 2016), others have shown weaker effects, especially when combined with toxic medications (Hou et al., 2023). Variations in dosage, timing, and duration of treatment may exist. However, CoQ10 appears to be promising in protecting the ovaries from damage caused by oxidative stress (Özcan et al., 2016; Xu et al., 2018).

This study was designed to explore how CP influences serum AMH levels in rats and to determine whether CoQ10 supplementation can modulate these effects. By doing so, we aim to shed light on possible strategies to reduce chemotherapy-related ovarian damage and help preserve fertility.

Materials and Methods

Experimental Animals

The Van Yüzüncü Yıl University Animal Experiments Ethics Committee approved this study (Approval No: 2025/06-12, dated May 29, 2025). The experimental rats were provided by the university's Experimental Medicine Application and Research Center. Thirty-five healthy female Wistar Albino rats (Rattus norvegicus) were used in the study. All rats were 3-4 months old and weighed between 200-250 grams. Rats were kept under standard laboratory conditions, with temperature 22-24°C, humidity between 55-60% and a 12 hours light/darkness program. They had unrestricted access to food and water throughout the experiment.

Experimental Groups and Treatment Protocols

There were five groups and seven rats in each group in the study. All substances were administered orally via gavage once daily at the same time for four consecutive weeks. Cyclophosphamide was dissolved in 0.5 mL of distilled water, while Coenzyme Q10 was suspended in 0.5 mL of olive oil. CP and CoQ10 application frequency and dose were determined according to Koşal et al.; (2023). The treatment groups were organized as follows: Control Group (n=7): Received 0.5 mL of physiological saline solution (0.9% NaCl) daily. Sham Group (n=7): Received 0.5 mL of olive oil daily. Cyclophosphamide (CP) Group (n=7): Received 6 mg/kg/day of CP (ENDOXAN® 50 mg tablet, Eczacıbaşı, Türkiye) in solution form. Coenzyme Q10 (CoQ10) Group

(n=7): Received 2.8 mg/kg/day of CoQ10 (Ocean®, Germany). CP + CoQ10 Group (n=7): Received both CP (6 mg/kg/day) and CoQ10 (2.8 mg/kg/day) concurrently.

Blood Sampling and AMH Measurement

At the end of treatment, all animals were deeply anesthetized (50 mg/kg ketamine + 10 mg/kg xylazine intramuscularly). After complete loss of pedal and corneal reflexes, euthanasia was performed by exsanguination by cardiac puncture in accordance with ethical standards. The collected blood was centrifuged at 3000 rpm for 10 minutes, and the serum was separated. Serum samples were stored at -80°C until the day of analysis. Serum AMH concentrations were measured using a commercial ELISA kit (Beckman Coulter AMH Gen II ELISA, USA) according to the manufacturer's instructions.

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics software (version 25.0; IBM Corp., Armonk, NY, USA). Since the AMH data did not meet the assumptions of normality, non-parametric methods were chosen for comparisons between groups. Initially, the Kruskal-Wallis H test was used to evaluate differences in AMH levels across all treatment groups. When significant differences were detected, pairwise comparisons were carried out using the Mann-Whitney U test. Results are expressed as mean ± standard deviation (SD), and a p-value below 0.05 was considered indicative of statistical significance.

Results

Serum AMH levels for the five experimental groups are presented in Table 1 as mean ± SD. CoQ10 group showed significantly higher AMH levels compared to the Control (p=0.0070), Sham (p=0.0262), CP (p=0.0006), and CP + CoQ10 (p=0.0023) groups. No statistically significant differences were observed between the remaining group pairs (p>0.05). The group that received CoQ10 alone exhibited the highest AMH concentration (6.22±0.97 ng/mL), supporting the notion that CoQ10 may have a protective effect on ovarian reserve. In contrast, the AMH levels observed in the CP group (4.09±0.67 ng/mL) and the CP + CoQ10 group (4.47±0.57 ng/mL) were lower, but these decreases did not reach statistical significance when compared to the control group. These findings suggest that although cyclophosphamide may exert adverse effects on ovarian reserve, such effects appear to be limited under the conditions of this study. A graphical representation of serum AMH concentrations is shown in Figure 1 as a box-and-whisker plot.

Table 1. Mean ± SD values of measured parameter and statistical groupings

	Control (n:7)	**Sham (n:7)**	**CP (n:7)**	**CoQ10 (n:7)**	**CP + CoQ10 (n:7)**
AMH (ng/ml)	4.67 ± 1.01[a]	5.01 ± 0.68[a]	4.09 ± 0.67[a]	6.22 ± 0.97[b]	4.47 ± 0.57[a]

Groups not sharing the same superscript letter ([a], [b]) are significantly different (p<0.05).

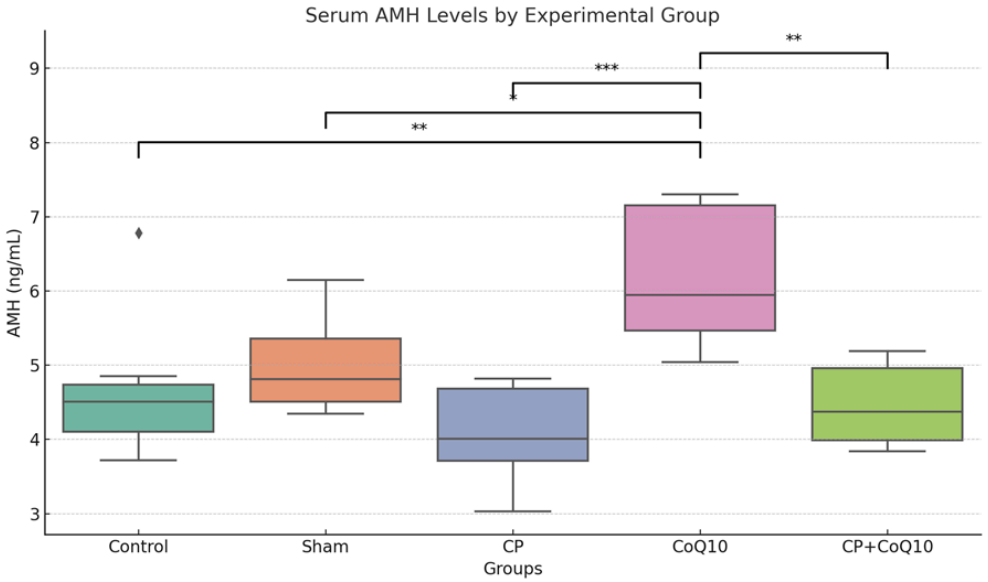

Figure 1. Serum AMH levels (ng/mL) in control, sham, cyclophosphamide (CP), Coenzyme Q10 (CoQ10), and CP+CoQ10 groups. Statistically significant differences were marked on the figure based on p-values as follows: Control vs CoQ10 → p=0.007 → **, CP vs CoQ10 → p=0.0006 → ***, CoQ10 vs CP + CoQ10 → p=0.0023 → **, p<0.05 was considered statistically significant (*)

Discussion

In this study, we examined the effects of CP on serum AMH levels in female rats administered CP. We also investigated whether CP combined with CoQ10 might provide any protective effect. Our findings showed that AMH concentrations were highest in the group receiving CoQ10 alone, suggesting that CoQ10 plays a supportive role in maintaining ovarian reserve. In contrast, CP alone did not cause a statistically significant decrease in AMH levels, although a numerical decrease was noted compared to the control group.

CP is an alkylating chemotherapeutic agent and, at certain doses, exhibits immunosuppressive properties (Ahlmann & Hempel, 2016). CP is widely used in the treatment of various cancers. It is also associated with reproductive toxicity, particularly affecting female germ cells (Barton et al., 2003). Infertility due to ovarian toxicity is a well-documented complication in women exposed to CP. Recent evidence suggests that CP triggers early apoptotic events, leading to a significant reduction in granulosa cell populations, impaired estradiol secretion, and extensive gonadotoxic effects (Hao et al., 2024). Some studies have shown that CP can reduce follicle counts and serum AMH concentrations (Ghosh et al., 2002; Hassanzadeh-Taheri et al., 2016). However, a study conducted with female Wistar Albino rats found that CP administration significantly reduced secondary and antral follicle numbers, while AMH levels were not affected (Türk, 2016). In our present study, only a minimal decrease in AMH concentrations was observed in the CP-treated group, and this decrease was not statistically significant. This result supports the results obtained by Türk (2016). Differences between studies may be due to differences in CP dose and exposure duration, which may not be sufficient to cause measurable changes in AMH levels. In particular, Türk (2016) emphasized that histological damage to ovarian follicles was evident even though AMH levels were unchanged. Similarly, our findings suggest that AMH alone may not fully reflect early ovarian damage, but histopathological evaluation of ovarian tissue may provide more informative information. Future studies that include histological analysis will be valuable for a more comprehensive understanding of CP-induced ovarian toxicity.

CoQ10 is an important endogenous lipid-soluble antioxidant that plays a role in mitochondrial respiratory chain activity. Its effects include contributing to ATP synthesis, neutralizing free radicals, and providing cellular protection against oxidative stress (Ben-Meir et al., 2015; Hidalgo-Gutiérrez et al., 2021). In a study conducted in mice, researchers found that disruption of endogenous CoQ10 synthesis in oocytes caused a significant decrease in the number of ovulating oocytes after puberty. While this may be due to a decrease in ovarian reserve, they also found that CoQ10 supplementation preserved follicular reserve, improved mitochondrial function in oocytes, and improved ovulatory outcomes (Ben-Meir et al., 2015). In our study, rats treated with CoQ10 alone exhibited significantly higher serum AMH concentrations compared to both the control and CP-treated groups. These findings support previous reports suggesting that CoQ10 supplementation may not only support follicular health but also delay ovarian aging (Ben-Meir et al., 2015; Özcan et al., 2016).

Interestingly, co-administration of CP and CoQ10 failed to produce a significant improvement in AMH levels compared to CP alone. While CoQ10 alone increased AMH levels, its combination with CP did not result in a similar increase, suggesting that the gonadotoxic effects of CP may have masked the beneficial effects of CoQ10. Similar challenges have been highlighted in other studies, where antioxidant therapy failed to completely counteract the adverse effects of chemotherapeutic agents on the ovaries (Zan & Duran, 2020). It is also worth noting that the pharmacokinetics, dosage, and timing of CoQ10 administration can significantly impact its efficacy. Studies have shown that earlier initiation, longer administration, or higher doses of CoQ10 may yield better results (Rodríguez-Varela & Labarta, 2021; Hou et al., 2023). This highlights the need for further research to optimize treatment protocols. Future studies should focus on determining the most effective dose and timing of CoQ10 supplementation, as well as investigating its long-term effects on fertility outcomes.

Conclusion

In conclusion CP treatment does not appear to adversely affect AMH levels, whereas CoQ10 supplementation significantly increases AMH concentrations. Notably, this beneficial effect of CoQ10 is abolished when it is co-administered with cyclophosphamide, suggesting that the gonadotoxic effects of CP may override the protective properties of CoQ10. Based on our findings, we propose that CoQ10 may contribute to the preservation of ovarian follicular reserve; however, it may be insufficient to fully prevent CP-induced ovarian damage. Further studies are warranted to more comprehensively evaluate the therapeutic potential of CoQ10 in preserving reproductive function, including investigations involving extended treatment durations, varying dosages, and optimized administration protocols.

Conflict of interest: The author has no conflicts of interest to declare.

Authors' Contributions: MY: Conceptualization, Funding acquisition, Data curation, Investigation, Methodology, Project administration, Software, Resources, Validation; Visualization, Writing-original draft, Writing-review & editing, Supervision and data analysis. Author interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

Ethics Committee Approval: This study was conducted at Van Yuzuncu Yil University Animal Hospital. This research was approved by Van Yuzuncu Yil University Animal Experiments Local Ethics Committee with a document that does not require approval for experimental animals (Date: 29/05/2025; Decision No: 2025/06-12)

Conflict Of Interest Statement: The author reports no conflicts of interest related to this work.

Data Availability Statement: The data supporting the findings of this study are available from the authors upon reasonable request after publication of the article. Corresponding Author Contact: mehmetyildiz@yyu.edu.tr

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

Ahlmann, M., & Hempel, G. (2016). The effect of cyclophosphamide on the immune system: implications for clinical cancer therapy. Cancer chemotherapy and pharmacology, 78(4), 661-671. https://doi.org/10.1007/s00280-016-3152-1

Barton, T. S., Wyrobek, A. J., Hill, F. S., Robaire, B., & Hales, B. F. (2003). Numerical chromosomal abnormalities in rat epididymal spermatozoa following chronic cyclophosphamide exposure. Biology of reproduction, 69(4),1150-1157. https://doi.org/10.1095/biolreprod.103.016261

Ben-Meir, A., Burstein, E., Borrego-Álvarez, A., et al., (2015). Coenzyme Q10 restores oocyte mitochondrial function and fertility during reproductive aging. Aging Cell, 14(5):887–895. https://doi.org/10.1111/acel.12368

Bentov, Y., Hannam, T., Jurisicova, A., Esfandiari, N., & Casper, R. F. (2014). Coenzyme Q10 supplementation and oocyte aneuploidy in women undergoing IVF-ICSI treatment. Clinical Medicine Insights: Reproductive Health, 8, CMRH-S14681. https://doi.org/10.4137/CMRH.S14681

Emadi, A., Jones, R. J., & Brodsky, R. A. (2009). Cyclophosphamide and cancer: golden anniversary. Nature reviews Clinical oncology, 6(11), 638-647. https://doi.org/10.1038/nrclinonc.2009.146

Gephart, B. D., Coulter, D. W., & Solheim, J. C. (2025). Effects of the Alkylating Agent Cyclophosphamide in Potentiating Anti-Tumor Immunity. International Journal of Molecular Sciences, 26(13), 6440. https://doi.org/10.3390/ijms26136440

Ghosh, D., Das, U. B., Ghosh, S., Mallick, M., & Debnath, J. (2002). Testicular gametogenic and steroidogenic activities in cyclophosphamide treated rat: a correlative study with testicular oxidative stress. Drug and chemical toxicology, 25(3), 281-292. https://doi.org/10.1081/DCT-120005891

Hao, X., Reyes Palomares, A., Anastácio, A., Liu, K., & Rodriguez-Wallberg, K. A. (2024). Evidence of apoptosis as an early event leading to cyclophosphamide-induced primordial follicle depletion in a prepubertal mouse model. Frontiers in Endocrinology, 15, 1322592. https://doi.org/10.3389/fendo.2024.1322592

Hassanzadeh-Taheri, M., Hosseini, M., Hassanpour-Fard, M., Ghiravani, Z., Vazifeshenas-Darmiyan, K., Yousefi, S., & Ezi, S. (2016). Effect of turnip leaf and root extracts on renal function in diabetic rats. Oriental Pharmacy and Experimental Medicine, 16(4), 279-286. https://doi.org/10.1007/s13596-016-0249-3

Hidalgo-Gutiérrez, A., González-García, P., Díaz-Casado, M. E., Barriocanal-Casado, E., López-Herrador, S., Quinzii, C. M., & López, L. C. (2021). Metabolic Targets of Coenzyme Q10 in Mitochondria. Antioxidants, 10(4), 520. https://doi.org/10.3390/antiox10040520

Hou, S., Tian, Z., Zhao, D., Liang, Y., Dai, S., Ji, Q., ... & Yang, Y. (2023). Efficacy and Optimal Dose of Coenzyme Q10 Supplementation on Inflammation-Related Biomarkers: A GRADE-Assessed Systematic Review and Updated Meta-Analysis of Randomized Controlled Trials. Molecular Nutrition & Food Research, 67(13), 2200800. https://doi.org/10.1002/mnfr.202200800

Jeelani, R., Khan, S. N., Shaeib, F., Kohan-Ghadr, H. R., Aldhaheri, S. R., Najafi, T., ... & Abu-Soud, H. M. (2017). Cyclophosphamide and acrolein induced oxidative stress leading to deterioration of metaphase II mouse oocyte quality. Free Radical Biology and Medicine, 110, 11-18. https://doi.org/10.1016/j.freeradbiomed.2017.05.006

Koşal, V., Rua, İ., Yüksek, V., & Keleş, Ö. (2023). Investigation of the effect of Coenzyme–Q10 on Cyclophosphamide induced testicular damage in male rats Investigación del efecto de la coenzima Q10 sobre el daño testicular inducido por ciclofosfamida en ratas macho. Revista Cientifica de la Facultad de Veterinaria, 33(2). http://doi.org/10.52973/rcfcv-e33278

La Marca, A., & Volpe, A. (2006). Anti-Müllerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool?. Clinical endocrinology, 64(6), 603-610. https://doi.org/10.1111/j.1365-2265.2006.02533.x

Özcan, P., Fıçıcıoğlu, C., Kizilkale, O., Yesiladali, M., Tok, O. E., Ozkan, F., & Esrefoglu, M. (2016). Can Coenzyme Q10 supplementation protect the ovarian reserve against oxidative damage?. Journal of assisted reproduction and genetics, 33(9), 1223-1230. https://doi.org/10.1007/s10815-016-0751-z

Rodríguez-Varela, C., & Labarta, E. (2021). Does coenzyme Q10 supplementation improve human oocyte quality?. International journal of molecular sciences, 22(17), 9541. https://doi.org/10.3390/ijms22179541

Rose, D. P., & Davis, T. E. (1977). Ovarian function in patients receiving adjuvant chemotherapy for breast cancer. The Lancet, 309(8023), 1174-1176. https://doi.org/10.1016/S0140-6736(77)92716-7

Türk P. (2016). Siklofosfamide Bağlı Ovaryan Toksisiteyi Azaltmada Silymarinin Rolü. Specialization Thesis, Faculty of Medicine, Uludag University, Bursa, Türkiye.

Villalba, J. M., & Navas, P. (2000). Plasma membrane redox system in the control of stress-induced apoptosis. Antioxidants & redox signaling, 2(2), 213-230. https://doi.org/10.1089/ars.2000.2.2-213

Weenen, C., Laven, J. S., Von Bergh, A. R., Cranfield, M., Groome, N. P., Visser, J. A., ... & Themmen, A. P. (2004). Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Molecular human reproduction, 10(2), 77-83. https://doi.org/10.1093/molehr/gah015

Xu, Y., Nisenblat, V., Lu, C., Li, R., Qiao, J., Zhen, X., & Wang, S. (2018). Pretreatment with coenzyme Q10 improves ovarian response and embryo quality in low-prognosis young women with decreased ovarian reserve: a randomized controlled trial. Reproductive Biology and Endocrinology, 16(1), 29. https://doi.org/10.1186/s12958-018-0343-0

Yildiz, M., Koca, D., Kilic Yildiz, B. N., Isik, M. Z., Turgut, A. O., & Çetin, Y. (2025). The Role of Anti-Müllerian Hormone in Van Cats in Relation to Sex and Reproductive Status. Veterinary Medicine and Science, 11(4), e70502. https://doi.org/10.1002/vms3.70502

Zan, E., & Duran, B. (2020). DHEA Supplementation Prevents Follicle Depletion due to Chemotherapy-Induced Ovarian Damage. EC Endocrinology and Metabolic Research, 5, 01-04.