Clinical Investigation / Araştırma

DOI: 10.4274/tjod.galenos.2024.89439

Turk J Obstet Gynecol 2024;21:175-9

Negative effects of ethanol on ovarian reserve and endometrium thickness: An animal study

Etanolün yumurtalık rezervi ve endometrium kalınlığı üzerine olumsuz etkileri: Bir hayvan çalışması

Enes Karaman[1], Mehmet Emin Ayağ[2]

[1]*Niğde Ömer Halisdemir University Faculty of Medicine, Department of Obstetrics and Gynecology, Niğde, Turkey*
[2]*Mehmet Emin Ayağ, Obstetrics and Gynecology Clinic, Mardin, Turkey*

Abstract

Objective: This study aimed to assess the effect of ethanol on the ovarian reserve and endometrium of rats by evaluating anti-Müllerian hormone (AMH) levels and follicle counts.

Materials and Methods: We performed histological follicle counting and AMH measurements to evaluate ovarian reserve. The study included 16 Wistar albino rats evenly distributed into two groups of eight rats each. The rats in the intervention group (group 1) were administered ethanol at a daily dose of 2.5 g/kg via oral gastric lavage for 30 days, whereas the control group (group 2) received water as a placebo via oral gastric lavage for the same period. At the end of 30 days, the animals were sacrificed, and 2 mL blood samples were collected for AMH measurements. Laparotomy was performed to remove the ovaries and uterus.

Results: Despite the lack of a meaningful distinction in the quantity of primordial and primary follicles between the two groups, a substantial disparity was observed in the overall follicle count and AMH levels. Specifically, the intervention group exhibited significantly lower total follicle counts and AMH levels than the control group ($p \leq 0.001$). The researchers also found that the endometrium of ethanol-treated rats was significantly thinner than that of control rats ($p \leq 0.001$).

Conclusion: This study concluded that ethanol consumption can negatively affect reproductive ability and the success of in vitro fertilization treatment by reducing ovarian reserve and thinning the endometrium.

Keywords: Ethanol, endometrium, ovarian reserve, rat

Öz

Amaç: Bu çalışmada, anti-Müllerian hormon (AMH) düzeyleri ve folikül sayıları değerlendirilerek etanolün sıçanların ovaryan rezervi ve endometriyumu üzerindeki etkisinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntemler: Araştırmacılar ovaryum rezervini değerlendirmek için histolojik folikül sayımı ve AMH ölçümleri gerçekleştirmiştir. Çalışmaya her biri sekiz sıçandan oluşan iki gruba eşit olarak dağıtılmış 16 Wistar albino sıçan dahil edilmiştir. Müdahale grubundaki sıçanlara (grup 1) 30 gün boyunca oral gastrik lavaj yoluyla günlük 2,5 g/kg dozunda etanol uygulanırken, kontrol grubuna (grup 2) aynı süre boyunca oral gastrik lavaj yoluyla plasebo olarak su verilmiştir. Otuz günün sonunda hayvanlar sakrifiye edilmiş ve AMH ölçümü için 2 mL kan örneği alınmıştır. Yumurtalıkları ve uterusu çıkarmak için laparotomi yapıldı.

Bulgular: İki grup arasında primordial ve primer folikül miktarında anlamlı bir fark olmamasına rağmen, genel folikül sayısı ve AMH seviyelerinde önemli farklılıklar vardı. Spesifik olarak, müdahale grubunda toplam folikül sayısı ve AMH seviyeleri kontrol grubuna kıyasla önemli ölçüde daha düşüktü ($p \leq 0,001$). Araştırmacılar ayrıca etanol ile tedavi edilen sıçanların endometriyumunun kontrol sıçanlarına göre önemli ölçüde daha ince olduğunu tespit etmiştir ($p \leq 0,001$).

PRECIS: Ethanol reduces ovarian reserve and endometrial thickness in rats, lowering AMH levels and total follicle counts, thereby negatively affecting reproductive health.

Address for Correspondence/Yazışma Adresi: Asst. Prof. Enes Karaman MD,
Niğde Ömer Halisdemir University Faculty of Medicine, Department of Obstetrics and Gynecology, Niğde, Turkey
Phone: +90 538 063 38 38 **E-mail:** dr.eneskaraman@gmail.com **ORCID ID:** orcid.org/0000-0003-1459-0606
Received/Geliş Tarihi: 31.07.2024 **Accepted/Kabul Tarihi:** 20.08.2024

Sonuç: Çalışma, etanol tüketiminin yumurtalık rezervini azaltarak ve endometriyumu incelterek üreme yeteneğini ve in vitro fertilizasyon tedavisinin başarısını olumsuz etkileyebileceği sonucuna varmıştır.

Anahtar Kelimeler: Etanol, endometriyum, yumurtalık rezervi, sıçan

Introduction

Alcoholism has been shown to have a negative impact on the female reproductive system, leading to amenorrhea, ovulation disorders, premature menopause, spontaneous abortion, and infertility[1,2]. Studies have also shown that ovarian reserve is reduced by alcohol consumption[1]. Although the consequences of ethanol exposure on the reproductive system of females have been meticulously documented, the specific mechanisms of alcohol's mechanism of action on the ovarian reserve and endometrium are not well understood.

Recent research indicates that ethanol suppresses the luteinizing hormone through its action on the hypothalamus, and chronic ethanol exposure has been associated with increased levels of serum estradiol[3]; however, the evidence regarding the effects of ethanol on peripheral reproductive tissues and their physiology is conflicting.

The ovarian reserve has been suggested to clearly indicate the fertility status of a woman as it has been demonstrated that reproductive capability is directly proportional to ovarian reserve. Due to these findings, many methods for determining ovarian reserve have been identified in the literature. Among these, the total ovarian follicle count and the measurement of anti-Müllerian hormone (AMH) levels can be used to predict ovarian reserve; consequently, these tests have become the most widely used methods for assessing ovarian reserve[4].

The primary aim of this study was to evaluate the effect of ethanol on AMH levels and ovarian reserve, as determined by histological analysis in a rat model. We also aimed to demonstrate the negative effects of alcohol consumption on reproductive ability.

Materials and Methods

Sixteen female Wistar albino rats weighing 180-210 grams and five to six months old were used in this study. All stages and procedures of this research were conducted in the Guinea Pig Experimental Animal Laboratory. During the experiments, the researchers adhered to strict animal care and use guidelines approved by the Institutional Review Board. All rats were maintained at 22±2 °C and subjected to a 12-h/12-h light/dark cycle without restriction of food or water.

Sixteen Wistar albino rats were randomly divided into two groups of eight rats each. Rats in the experimental group (group 1) were administered 2.5 g/kg ethanol daily via oral gastric lavage for 30 days. Rats in the control group (group 2) were given water as a placebo for the same period and using the same method. The rats eliminated ethanol at approximately 7.9 mmol/kg h[5]. To prevent possible effects of blood ethanol levels on AMH measurements in the study group, laparotomy was performed in both groups on the day after the last ethanol administration. A total of 2 mL of intracardiac blood was taken to evaluate AMH, and the ovaries and uteri of all animals were obtained with immediate laparotomy. Ovarian follicle counts were taken according to types (primordial, primary, secondary, tertiary, and total) in all rats and endometrial thickness was histologically evaluated.

After 30 days, 2 mL of intracardiac blood was taken to evaluate AMH, and the ovaries and uteri of all animals were obtained with immediate laparotomy. Ovarian follicle counts were taken according to types (primordial, primary, secondary, tertiary, and total) in all rats, and endometrial thickness was histologically evaluated.

Before laparotomy, a 2 mL blood sample was obtained via intracardiac aspiration after appropriate anesthesia. Blood serum was separated by centrifugation at 1000 x g for 15 min at 4 °C. Serum samples were stored at 20 °C until assayed. AMH levels (ng/mL) were determined using a BioTek Synergy HT Microplate Reader (USA) capable of measuring absorbance at 450 nm, which was used according to the manufacturer's instructions. In this procedure, a Cusabio Rat AMH kit was used, and the correction wavelength was set to 600 nm-630 nm by a blinded researcher in a private medical laboratory. All samples were tested on the same plate in triplicate measurements. The minimum detection limit of the AMH enzyme-linked immunosorbent assay kit was 0.051 nanograms per milliliter (ng/mL), and the inter-assay and intra-assay variations were 15%.

5 µm thick slices of ovarian sections and five randomly selected samples from each ovary were used to assess follicular activity. Hematoxylin-eosin staining was performed, and a pathologist evaluated the samples using a light microscope and a blinded method. All follicles were counted and categorized into primordial, primary, secondary, and tertiary stages, according to standard microscopic anatomy textbooks. The ovarian reserve was determined by summing these four categories.

The endometrial thickness (nm) was also measured, and 100 cells were evaluated in each section. In addition, each tenth section of 100 slices was examined at 400X magnification to count the number of glands in the area.

Before the start of the study, written approval was obtained from the Experimental Animals Local Ethics Committee of the Faculty of Medicine of Niğde Ömer Halisdemir University (approval number: 2024/10; date: 31.05.2024).

Statistical Analysis

For statistical analyses, the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software version 15.0 was used. Normality tests, including the Shapiro-Wilk test,

were conducted to assess whether the variables followed a normal distribution. If a variable failed to show a normal distribution, the Mann-Whitney U test was employed. Variables with a normal distribution were analyzed using the t-test. The significance level was set at $p<0.05$. The results are expressed as the mean ± standard deviation, and power analysis of the values was performed using Sigma-Aldrich 3.5 software, as indicated below the tables.

Results

Both groups showed comparable numbers of primordial and primary follicles; however, the overall follicle count in the study group was notably lower than that in the control group (12.10±4.94 vs. 28.60±6.80, p≤0.001). Moreover, AMH levels were considerably lower in the ethanol group than in the control group (24.55±28.03. 91.38±26.54, p≤0.001). Furthermore, endometrial thickness was significantly lower in the ethanol group than in the control group (487±53 vs. 312±58, p≤0.001). All measured parameters are summarized in Table 1, Figure 1, and Figure 2, and endometrial thickness comparisons between the groups are illustrated in Figure 3.

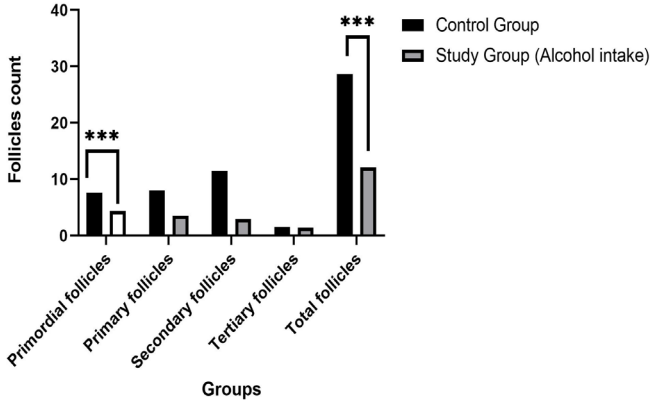

Figure 1. Bar graphs of the follicle count
***: p<0.01

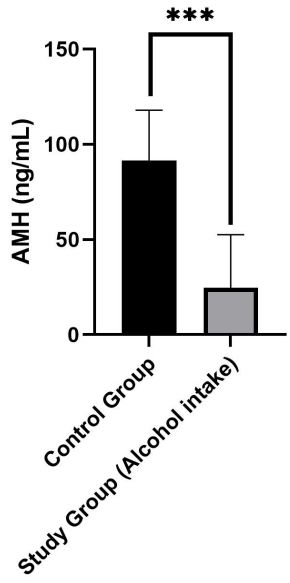

Figure 2. Bar graph of AMH levels
***: p<0.01
AMH: Anti-Müllerian hormone

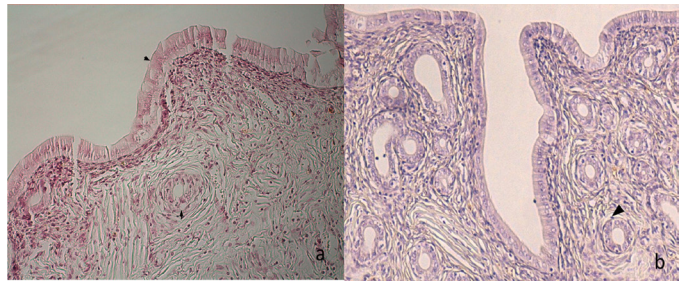

Figure 3. Comparison of endometrial histology
a. Histological examination of the control group (H&E)(x400)
b. Histological examination of the alcohol intake (H&E)(x400)
H&E: Hematoxylin-eosin

Table 1. Results of ovarian follicule tests and endometrium thickness of the groups

Parameters	Control group	Study group (alcohol intake)	p
Primordial follicles	7.63±4.86	4.30±2.65	0.108
Primary follicles	8.02±3.54	3.55±1.60	0.039
Secondary follicles	11.47±3.11	2.90±2.41	<0.001
Tertiary follicles	1.55±0.75	1.41±0.74	0.74
Total follicles	28.60±6.80	12.10±4.94	<0.001
AMH (ng/mL)	91.38±26.54	24.55±28.03	<0.001
Endometrium (nm)	487±53	312±58	<0.001

AMH: Anti-Müllerian hormone

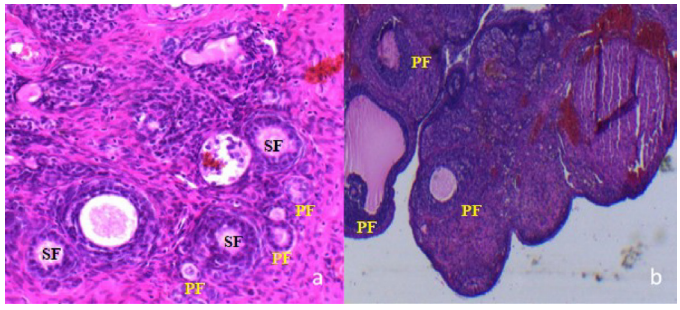

Figure 4. Comparison of ovarian histology (SF; Secondary follicle, PF; Primordial follicle)

a. Histological examination of the control group (H&E)(x400)

b. Histological examination of the alcohol intake (H&E)(x400)

H&E: Hematoxylin-eosin

Figure 4 displays a histological image that exhibits evident changes in the ovaries of ethanol-treated rats compared with the control group.

Discussion

Alcohol has been proven to have detrimental effects on the neuroendocrine, cardiovascular, and immune systems, and this is a widely accepted fact[6,7]. Despite the known adverse effects of ethanol on the female reproductive system, few studies have focused on the toxicity of alcohol to specific tissues[8]. Therefore, identifying the influence of ethanol on various organs of the female reproductive system is an important research focus, especially since available studies have shown varying degrees of alcohol-induced damage.

As mentioned previously, ovarian reserve determination has become a central part of reproductive assessment in females. The measurement of AMH levels is a good proxy for ovarian reserve[9,10]. This has been demonstrated through evidence obtained from various studies[11]. Thus, we utilized AMH measurements to determine and compare the ovarian reserve of rats in this study. We also directly counted the number of follicles via histological evaluation of tissues.

Normally, the highest levels of AMH expression are observed in the granulosa cells of secondary follicles and preantral and small antral follicles[12]. When this finding is evaluated considering a study by Chuffa et al.[13] which reported advanced atresia of secondary, antral, and preovulatory follicles in the granulosa layer of rats exposed to ethanol, it seems that the mechanism of AMH reduction is associated with the damage to the granulosa layer by ethanol. However, this may not be the only explanation, as other mechanisms may contribute to the overall reduction in AMH levels associated with alcohol consumption. In this study, using a prospective randomized rat model, we demonstrated that ethanol ingestion leads to decreased AMH levels and adverse effects on ovarian reserve. Although our results agree with those of especially controlled studies and some clinical studies[14,15], most clinical studies report opposing findings. For instance, a large population-based study by Dólleman et al.[16] failed to find any relationship between alcohol consumption and AMH levels according to age. This was also true in various studies of the same type conducted in several countries[17-20]. However, it is important to note that the measures used for female fertility varied from study to study, and not every study focused on alterations in AMH levels or other methods of laboratory analyses.

Chuffa et al.[13] investigated the effects of ethanol on the types and numbers of follicles. Their findings revealed that rats treated with ethanol experienced a decrease in the number of primordial follicles, with no noticeable effect on the number of primary or tertiary follicles.

Somewhat in contrast with these results, our findings demonstrated that the secondary and total follicle counts were significantly decreased in the rat group receiving ethanol. We also found that follicle counts were correlated with AMH levels in our study group.

Thin endometrium, which is often a result of a hypoestrogenic state, is significantly associated with implantation failure and pregnancy loss[21]. Lack of estrogen may be due to the lower number of follicles, which in turn leads to a vicious cycle. Studies suggesting the adverse effects of alcohol on reproductive health have shown poor pregnancy outcomes in those exposed to alcohol during pregnancy[22]. Despite the presence of convincing arguments for both sides of the debate, the adverse effects of alcohol use on the success of in vitro fertilization and the quality of embryos are widely accepted[23]. Considering that alcohol consumption may cause a thin endometrium, it is evident that the implantation of embryos may also suffer from alcohol use, both in the long and short term. Our results add to these adversities by suggesting that ovarian reserve is negatively affected by ethanol consumption in a rat model of daily alcohol consumption.

Study Limitations

Although we believe our results are convincing, it is important to consider that the metabolic differences between humans and rats, especially in terms of liver function, reproductive capabilities, and evolutionary differences, could explain the lack of agreement between our results and most clinical studies. These data are important to consider when evaluating the effects of ethanol on ovarian reserve.

Conclusion

To the best of our knowledge, this study is the first to evaluate ovarian reserves in rats exposed to ethanol for 30 days by analyzing AMH levels and histological features of ovarian follicles. In addition, this study clearly revealed that alcohol use was associated with decreased ovarian reserve and a thin endometrium based on histological findings.

Acknowledgments

The author would like to thank histologist Esra Balcıoğlu for her help.

Ethics

Ethics Committee Approval: Before the start of the study, written approval was obtained from the Experimental Animals Local Ethics Committee of the Faculty of Medicine of Niğde Ömer Halisdemir University (approval number: 2024/10; date: 31.05.2024).
Informed Consent: Not necessary.

Authorship Contributions

Surgical and Medical Practices: E.K., Concept: E.K., Design: E.K., M.E.A., Data Collection or Processing: M.E.A., Analysis or Interpretation: E.K., M.E.A., Literature Search: E.K., M.E.A., Writing: E.K.
Conflict of Interest: No conflict of interest was declared by the authors.
Financial Disclosure: The authors declared that this study received no financial support.

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