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ORIGINAL PAPER

Does isotretinoin affect spermatogenesis in the long term? A rat model

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Summary *Objectives: Spermatogenesis, in which cell regeneration continues, can be affected by environmental, chemical, psychological factors or various diseases. There is conflicting information in the literature about the effect of isotretinoin, which is widely used in acne treatment, on testes and spermatogenesis. Therefore, we planned a rat study to evaluate the long-term efficacy of oral isotretinoin on testicular tissues and spermatogenesis.*

Materials and methods: The Group 1 (n = 6) 7.5 mg/kg/day and the Group 2 (n = 6) received isotretinoin at a dose of 30 mg/kg/day dissolved in sunflower oil, the Sham Group (n = 6) received only sunflower oil by gavage, and the control group (n = 6) received standard feed and water for four weeks. After the 4th week, all animals were fed with standard feed and water and followed for the next four weeks. At the end of the 8th week, all animals were sacrificed under deep anesthesia. Seminiferous tubule diameters, epithelial thickness, apoptotic index, sperm number and motility recorded

Results: Sperm count, motility, vitality, diameter of seminiferous tubule and germinal epithelium thickness were decreased and apoptotic index increased in the groups received isotretinoin. There was no significant difference between the groups in terms of testosterone levels.

Conclusions: We consider that further comprehensive studies, including human clinical trials, should be conducted to examine the negative effects of isotretinoin on spermatogenesis in the long term especially when there is a need using isotretinoin in men for various reasons and to eliminate the contradictions in the literature in this regard.

KEY WORDS: *Isotretinoin; Rat; Spermatogenesis; Testis.*

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INTRODUCTION

Spermatogenesis is a complex process in which mature sperm cells are formed from spermatogonial stem cells as a result of mitosis, meiosis, and cell differentiation. In this active process, the cell cycle continues in seminiferous tubules, which can make the spermatogenetic process sensitive to various factors such as environmental, chemical, toxic and pharmacological agents. One of them is isotretinoin, which is widely used in the treatment of acne, one of the most common and disturbing diseases among young people. Isotretinoin is the only drug effective on all pathogenic mechanisms of acne, such as inflammation, increased sebum secretion, overgrowth of bacteria such as *Propionibacterium acnes*, and ductal hypercornification (1). Although oral isotretinoin is a very effective treatment, it has a teratogenic effect in pregnant women (2). However, there is controversial information on the effect of isotretinoin in men. While there are several studies on spermatogenesis, we have limited information on the long-term effectiveness of isotretinoin use on testicles and spermatogenesis. In addition, it may not be ethically appropriate to conduct studies for evaluating its histological effects in humans. As there is no clear information about the effects of isotretinoin on spermatogenesis, this situation can be a matter of concern and curiosity in male patients using the drug and physicians recommending this drug. Therefore, we planned a rat study to evaluate the long-term efficacy of oral isotretinoin on testicular tissues and spermatogenesis.

MATERIALS AND METHODS

Animals

The animals were procured from the *Samsun Experimental Animals Research and Ethical Application Center* (EARAC) after obtaining an ethics committee approval from the *Animal Studies Ethical Committee at Ordu University*. This study was performed in accordance to the Guiding Principles for the Care and Use of Laboratory Animals.

In our study, we used a total of 24 male Wistar-albino rats, 4-8 weeks old, with an initial weight of 250-300 g. The rats were housed in rooms at 22 ± 1°C and 45-55% humidity, ensuring 12-hour light and dark cycles and including a maximum of 3 rats in each cage. The temperature and humidity were measured by thermometer and hygrometer, respectively. The ventilation was provided by a room aspirator. The rats had free access to feed and water. All rats were fed with normal rat chow (*Nucleon, Ankara, Turkey*).

Formation of the groups and experimental design
A total of 24 male Wistar-albino rats, 4-8 weeks old, were used and randomly divided into 4 groups, including 6 in each group. The Group 1 (n = 6) received isotretinoin (*Roaccutane, Roche, Basel, Swiss*) at a dose of 7.5 mg/kg/day dissolved in sunflower oil, the Group 2 (n = 6) received isotretinoin at a dose of 30 mg/kg/day dissolved in sunflower oil, the Sham Group (n = 6) 6) received only sunflower oil by gavage, and the control group (n = 6) received standard feed and water for four weeks. After the 4th week, all animals were fed with standard feed and water and followed for the next four weeks. At the end of the 8th week, all animals were sacrificed by cervical dislocation under deep anesthesia with intraperitoneal of ketamine 90 mg/kg (*Ketalar; Eczacıbaşı, Istanbul, Türkiye*) and xylazine hydrochloride 3 mg/kg (*Rompun; Bayer, Leverkusen, Germany*). Their testicles were excised bilaterally and their epididymis and testicular tissues were separated from each other for analysis.

Biochemical analyses in serum
After being kept at room temperature for 30 minutes, the blood specimens placed into gel-containing tubes were centrifuged at 3000 g for 15 minutes. The serum samples separated from the centrifuged blood were stored at -80°C until analysis. Their testosterone, FSH and LH levels were measured after the serums were dissolved (*Cobas 8000 e 602, Hitachi, Roche autoanalyzer*).

Histological procedure
At the end of the experiment, while the animals were under deep anesthesia, a midline incision was made, and their testicular and epididymis tissues were meticulously separated from the surrounding tissues. Then their epididymis and testicular tissues were separated from each other for analysis. The epididymis tissues were placed in Tris phosphate buffer solutions and the testicular tissues in Bouin solutions for fixation after they were divided vertically into two pieces. After the tests were fixed in Bouin solution, the entire half tests were kept for 3 days in a dry environment protected from light. The tissues were then subjected to routine histological tissue follow-up procedures on the *Thermo Scientific Excelsior™ AS* (*Thermo Fisher Scientific, Waltham, MA USA*), an automatic tissue tracking device.
After the follow-up process, the tissues were blocked by embedding in paraffin in *HistoCore Arcadia H and HistoCore Arcadia C* (*Leica Biosystems Nussloch GmbH, Nussloch, Germany*).
Then, 5-µm thick sections were taken with a fully automatic microtome (*Leica RM 2255, Tokyo, Japan*). The sections were mounted on slides and stained with hematoxylin and eosin (*H & E, Leica autostainer XL, Minnesota, USA*) for histopathological examination and measurements of seminiferous tubule diameter and epithelial thickness. The seminiferous tubule diameter and epithelial thickness were measured using the Olympus DP 71 camera-mounted microscope (*Olympus, Tokyo, Japan*).
The Analysis 5 Research (*Olympus Soft Imaging Solution GmbH, Münster, Germany*) program was used for this purpose and the properties of the interstitial space. We used the same microscope to take photographs.

Epididymal sperm count, and sperm motility and vitality
The right epididymis was used to evaluate epididymal sperm count, sperm motility and viability. The epididymis was placed in a Petri dish with 2 ml of Tris buffer solution and divided into 4 parts. It was incubated at 37°C for 30 min to permit sperm release. Sperm count and motility were evaluated using the Makler sperm counting chamber (*Sefi -Medical Instrument, Haifa, Israel*). Five milliliters of sperm fluid were dropped from the homogenate into the center of the chamber, and the glass lid was closed. Thus, the sperms were allowed to swim in the area with a depth of 10 µm. The sperm count was performed by a light microscope at x 100 magnification. Percentages of the motile sperm were calculated among at least 100 spermatozoa. Then, to evaluate sperm viability, 5 µl of sperm sample was placed on slides, a drop of 1% eosin Y was added and closed with a coverslip. It was evaluated by counting 100 motile and immotile sperms in different areas at x 200 magnification. Orange colored sperms were considered dead, and the unstained ones were considered alive. The percentage of viable sperms was calculated (3).

TUNEL staining and apoptosis
Apoptosis was examined using TUNEL staining to detect DNA fragmentation in the seminiferous tubule epithelium. TUNEL staining was performed using the *In Situ Cell Death Detection Kit* (*Roche Diagnostics, Mannheim, Germany*). Evaluation of the TUNEL stained sections was performed using the light microscope at x 400 by an experienced histologist blinded to the identity of the specimens. TUNEL-positive cells with no necrotic areas and a brown nucleus were considered apoptotic. TUNEL-positive cell numbers in 10 seminiferous epithelium regions and the total number of cells were used to calculate AI (TUNEL-positive cells/total cells x 100) (4).

Statistical analysis
The data were tested for normality using the Shapiro-Wilk test and for homogeneity of variance using the Levene's test prior to the analyses. One-way ANOVA followed by Tukey as post-test was used to compare the groups. Descriptive statistics of the data set were expressed as means, standard deviations, and minimum and maximum values. A p-value less than 0.05 (two-sided) was considered statistically significant. All statistical analyses were performed using the SPSS v25 (*IBM Inc., Chicago, IL, USA*) statistical software.

Results
As a result of the analysis of variance for sperm motility, the difference between the groups was found to be statistically significant ($p < 0.001$). As a result of the Tukey test performed to determine different averages, the sperm motility rate in Group 1 and Group 2 was significantly lower than the control and Sham groups, but there was no significant difference between the control and Sham groups ($p > 0.05$) ($p < 0.05$). In addition, the decrease in sperm motility was higher in the Group 2 than in the Group 1.
As a result of the analysis of variance for sperm count, the

difference between the groups was found to be statistically significant ($p < 0.0$). As a result of the Tukey test, the sperm count was significantly lower in the Group 2 than in the control group ($p < 0.05$), but the differences between the other groups were not statistically significant ($p > 0.05$).

As a result of the analysis of variance for sperm vitality, diameter of seminiferous tubule, germinal epithelium thickness, and apoptotic index, the differences between the groups were found to be statistically significant ($p < 0.001$) (Table 1). As a result of the Tukey test performed to determine different averages; while there was no significant difference between the control and Sham groups ($p > 0.05$), the Groups 1 and 2 had significantly lower sperm vitality, diameter of seminiferous tubule ($p < 0.05$) and germinal epithelial thickness ($p < 0.05$) and higher apoptotic index value than the control and sham groups ($p < 0.05$) (Figure 1).

As a result of the variance analysis performed to compare the testosterone amounts of the groups, the difference between the group averages was not found to be statistically significant ($p > 0.05$).

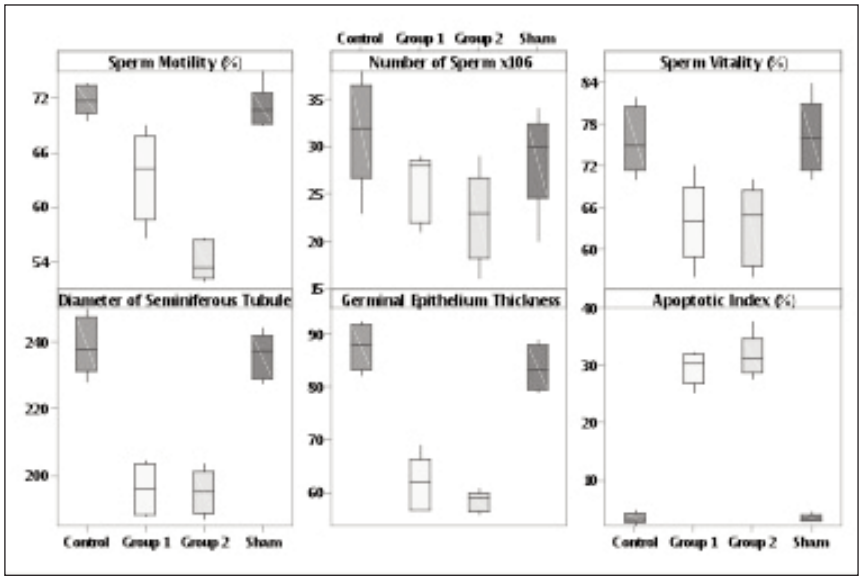
For all groups, FSH and LH values were below 0.10 mIU/ml, therefore it was not possible to compare their FSH and LH values. There was no statistically significant difference between the groups in terms of testosterone levels.

Table 1.
Descriptive statistics and the results of statistical analysis for the study variables.

Variables	Group	N	Mean	Std. Dev.	Min-Max	P-value
Sperm Motility (%)	Control	6	71.80a	1.59	69.56-73.68	< 0.001 (F = 51.24)
	Sham	6	71.10a	2.20	68.96-75.00	
	Group 1	5	63.43b	4.92	56.52-68.96	
	Group 2	6	53.85c	2.09	51.72-56.52	
Number of Sperm x10^6	Control	6	31.50a	5.58	23.00-38.00	< 0.05 (F = 3.67)
	Sham	6	28.67ab	5.05	20.00-34.00	
	Group 1	5	25.80ab	3.56	21.00-29.00	
	Group 2	6	22.67b	4.68	16.00-29.00	
Sperm Vitality (%)	Control	6	75.67a	4.63	70.00-82.00	< 0.001 (F = 10.20)
	Sham	6	76.33a	5.13	70.00-84.00	
	Group 1	5	64.00b	5.83	56.00-72.00	
	Group 2	6	63.67b	5.57	56.00-70.00	
Diameter of Seminiferous Tubule	Control	6	239.31a	9.02	228.42-252.81	< 0.001 (F = 60.75)
	Sham	6	236.32a	6.58	227.63-244.35	
	Group 1	5	196.27b	7.59	187.96-204.48	
	Group 2	6	195.34b	6.44	187.20-203.68	
Germinal Epithelium Thickness	Control	6	87.69a	4.43	82.14-92.64	< 0.001 (F = 75.92)
	Sham	6	83.74a	4.41	78.96-88.93	
	Group 1	5	61.73b	5.20	56.66-69.10	
	Group 2	6	58.58b	1.93	55.87-60.80	
Apoptotic Index (%)	Control	6	3.41b	0.93	2.25-4.84	< 0.001 (F = 263.47)
	Sham	6	3.49b	0.65	2.91-4.56	
	Group 1	5	29.78a	2.80	25.53-32.45	
	Group 2	6	31.94a	3.66	27.87-37.84	

F, one-way ANOVA.
According to Tukey's test, means that do not share a letter are significantly different ($p < 0.05$).

Figure 1.
Box-plot of the study variables in the groups.



Discussion

Isotretinoin is a synthetic retinoid used in the treatment of severe nodulocystic acne where other treatments have failed (5). Retinoids are also used to manage other dermatological conditions, such as rosacea, folliculitis, sarcoidosis, granuloma annulare, seborrheic dermatitis, and a variety of keratinization disorders (6). Although isotretinoin has been used for many years, hesitations about its side effects still exist. Both human and animal studies have reported that it has serious teratogenic effects, decreasing ovarian reserves after 6 months of use of isotretinoin in women (2, 7, 8). Given the wide clinical usage of retinoids, clarification of whether retinoids affect the reproductive system in male patients of childbearing age is urgently needed. In addition, there are conflicting results regarding its side effects on male reproductive system. Studies have argued that both deficiency and high levels of vitamin A suppress spermatogenesis (9).

Some other studies have suggested that vitamin A is necessary for spermatogenesis and its deficiency may cause defects in germ cell and testosterone production (10).

Some human studies argue that isotretinoin has a positive effect on sperm parameters, however we have reached the opposite results in our study. For example, *Cinar et al.* administered a cumulative dose of isotretinoin to 81 male patients to investigate the effects of systemic

isotretinoin on male fertility. They examined spermiogram parameters (sperm concentration, total progressive motility, total progressively motile sperm, normal morphology and viability) and total testosterone, FSH and LH levels before and after the treatment. They observed no significant change in the hormone profile after the treatment, but found significant positive changes in the spermiogram parameters (11). *Amory et al.* administered isotretinoin to patients between the ages of 21 and 60 years with impaired sperm parameters, and found no significant change in their sperm motility, but observed an increase in their sperm concentrations (12). On the other hand, some other human studies reported that isotretinoin treatment did not affect spermatogenesis and hypothalamic-pituitary-gonadal axis (13-15).

The reason for the different results in our study and those mentioned above may be due to the difference in molecular doses, various uncontrollable non-standard subjective factors that affect the sperm parameters of people with different physical factors such as environment and diet. The wide age range of participants or the investigation of people with damaged spermatogenesis may be the reason for this difference alone. In addition, due to the nature of the research and ethical restrictions in these human studies, it was tried to have an idea by looking at very limited sperm parameters. Apart from this, most of these studies in humans do not have a placebo and control groups. Our study is valuable as it provides objective histopathological data with peers raised in controlled environments together with standard environment and environmental factors. So much so that at the end of our study, we found that isotretinoin reduced sperm motility, viability and numbers, decreased germinal epithelial thickness and seminiferous tubule diameters, and impaired spermatogenesis by stimulating apoptotic index in testicles. Also, interestingly, despite studies claiming that synthetic retinoids may have beneficial effects on semen parameters, isotretinoin has never been offered as a treatment option in patients with impaired spermiogram parameters (16).

Sengör et al. examined the effects of another synthetic retinoid, acitretin, on spermatogenesis, reported no significant change in the spermatogenesis evaluation and mean tubular diameter between the acitretin-treated and control groups, and found that acitretin had no effect on spermatogenesis (17). We found the opposite results in our study and reached objective histopathological data showing that retinoids stimulate the apoptotic index. This difference may be due to dose-dependence or because Sengör et al. could not control some valuable parameters, including the apoptotic index.

Apoptosis is a complex event regulated by the balance of inducer and repressor factors. It has a critical role in the elimination of damaged spermatogonial cells to prevent the production of abnormal sperm cells (18).

Spermatocytes that cannot complete their mitotic division are eliminated by apoptosis (19). Apoptosis in germ cells increases in infertile men (20, 21). Our study found significantly higher apoptotic index in both the groups 1 and 2 than in the control group. This increase in the apoptotic index may be a natural regulation to protect against possible teratogenicity. In addition, although the exact mechanism of action of retinoids is not known, they stimulate apoptosis in sebaceous glands and disrupt cell cycle arrest (22, 23).

Spermatogenesis is an active process in testis, including continuous cell cycle. It is a natural result that systemic isotretinoin acts on the seminiferous tubules, where cell division continues, along with the sebaceous glands. We consider that this may be the reason for the deterioration in sperm quality and the increase in apoptotic index in our study. Like our study, a study examined the apoptotic index and suggested that isotretinoin had damaging effects on spermatogenesis (24). Studies about the relationship between different molecules and spermatogenesis have shown an inverse relationship between apoptotic index and spermatogenesis, and this result is in line with those in our study (25, 26). The apoptotic index has not been examined in any of the studies suggesting that isotretinonin has a positive effect on spermatogenesis. If isotretinoin had a spermatogenesis stimulating effect as claimed, it should have decreased in the apoptosis index. However, such information is not mentioned in the presented studies.

In particular, the germinal epithelium in the seminiferous tubules is the testicular component directly related to spermatogenic activity (27). Therefore, germinal epithelial thickness is a useful parameter in evaluating sperm production (28). It decreases due to decreased cell division (29). Isotretinoin-induced cell cycle arrest may also be responsible for the decrease in germinal epithelial thickness, which is one of the results of our study, and therefore the decrease in sperm counts. In our study, isotretinoin decreased germinal epithelial thickness and seminiferous tubule diameters and sperm concentration in testis. These results suggest that isotretinoin has serious damaging effects on spermatogenesis, which is in line with the histopathological results in the literature indicating impaired spermatogenesis.

Sperm motility is an absolutely necessary function for male fertility. Although it has not been fully explained how the sperm gain this ability while passing through the epididymis, it is known that various signaling pathways are effective in developing this ability (30, 31). To be motile, human sperm need a morphologically complete flagellum, be able to produce energy to power flagellar movement and functional signaling pathways. Although our study did not reveal precisely which part of this system isotretinoin affects, it has clearly shown that isotretinoin decreases sperm motility in both groups using drugs.

The effect of environmental factors on spermatogenesis is well known. Several factors such as environmental toxins, diet, stress, drugs, frequency of ejaculation can affect sperm parameters (32, 33). In human studies, it is practically not possible to ensure that all subjects live under the same conditions. This may be the reason for contradictory results in studies. We consider that our results are more reliable since the feeding and environmental conditions of all animals were standard. We also think that our study is more objective, as it revealed the drug's negative effects on testicular tissue histologically (Figures 2, 3).

There are also conflicting results regarding the effects of isotretinoin on pituitary hormones. In their study with 47 patients, *Karadağ et al.* (22). reported that isotretinoin may have an effect on pituitary hormones. *Cinar et al.*, on

Figure 2.
Histological sections in rat testis stained with hematoxylin and eosin (H-E, ×400). Control group (C), sham group (S), 7.5 mg/kg isotretinoin and 30 mg/kg isotretinoin.

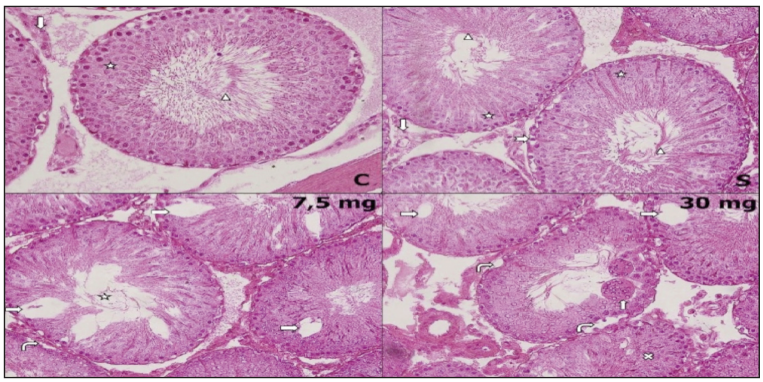
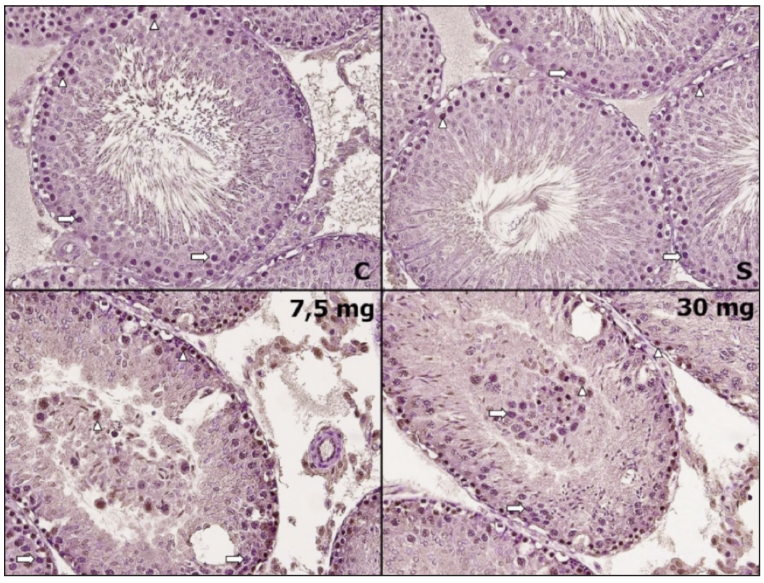


Figure 3.
Histological sections in rat testis stained with TUNEL (×400). Control group (C), sham group (S), 7,5 mg/kg isotretinoin and 30 mg/kg isotretinoin. Normal cells (right arrow) and apoptotic cells (arrowhead).
C: Seminiferous tubule (star) with regular germinal epithelium and normal structure observed, spermatozoa in the seminiferous tubule lumen (arrowhead), interstitial area with normal structure and Leydig cell (lower arrow);
S: Seminiferous tubule (star) with regular germinal epithelium and normal structure observed, spermatozoa in the seminiferous tubule lumen (arrowhead), occasional vacuolization around spermatogonium (dashed right arrow)
7.5 mg/kg: Loss or reduction of spermatozoa (star), vacuolar degeneration (right arrow), opening between seminiferous tubule basal membrane and germinal epithelium (right bent arrow).
30 mg/kg: Opening between seminiferous tubule basal membrane and germinal epithelium (right bent arrow), vacuolar degeneration (right arrow), giant cells with multiple nuclei (upper arrow), disorganization and degenerations of germinal epithelium in the seminiferous tubule (multiplication sign).



the other hand, reported no significant change in FSH, LH, and testosterone levels after the use of isotretinoin (12). Comparing the control group with the groups using 7.5 mg/kg and 30 mg/kg of isotretinoin, our study found no significant difference between their testosterone levels in the long term, but the FSH and LH levels were below measurable values in all groups. Therefore, it was not possible to compare the FSH and LH values. However, our results suggest that the effect of isotretinoin on testicles is due to its direct effect on the tissue rather than hormonal changes.

As a result, rat studies about the long-term effect of isotretinoin are limited and include contradictory results. Therefore, the inability to reach sufficient studies to be a benchmark for our study may be one of the limitations in our study. However, considering that the results obtained from rats subjected to the same environmental factors and feeding are supported by apoptotic index, parallel objective histopathological results and biochemical values, our study makes a valuable contribution to the literature.

Conclusions

Our study concludes that the negative effects of isotretinoin on spermatogenesis may be due to its direct damage to testicular tissues. In addition to the necessity of conducting human studies to evaluate the long-term effects of isotretinoin on testicles, we consider that further comprehensive studies, including human clinical trials, should be conducted to examine the negative effects of isotretinoin on spermatogenesis in the long term especially when there is a need using isotretinoin in men for various reasons, and to eliminate the contradictions in the literature in this regard.

References

1. Zouboulis CC, Bettoli V. Management of severe acne. Br J Dermatol. 2015; 172:27-36.

2. Henry D, Dormuth C, Winquist B, et al. Occurrence of pregnancy and pregnancy outcomes during isotretinoin therapy. CMAJ. 2016; 188:723-730.

3. Sankako MK, Garcia PC, Piffer RC, et al. Possible mechanism by which zinc protects the testicular function of rats exposed to cigarette smoke. Pharmacol Rep. 2012; 64:1537-1546.

4. Hancı H, Odacı E, Kaya H, et al. The effect of prenatal exposure to 900-MHz electromagnetic field on the 21-old-day rat testicle. Reprod Toxicol. 2013; 42:203-209.

5. Shahada OO, Kurdi AS, Aljawi AF, et al. Synovitis, Acne, Pustulosis, Hyperostosis, and Osteitis Syndrome Diagnosis in Adolescent and Isotretinoin as a Possible Serious Exacerbating Factor. Cureus. 2022; 14:e22776.

6. Motamedi M, Chehade A, Sanghera R, Grewal P. A Clinician's Guide to Topical Retinoids. J Cutan Med Surg. 2022; 26:71-78.

7. Mehra T, Borelli C, Burgdorf W, et al. Treatment of severe acne with low-dose isotretinoin. Acta Derm Venereol. 2012; 92:247-248.

8. Aksoy H, Cinar L, Acmaz G, et al. The effect of isotretinoin on ovarian reserve based on hormonal parameters, ovarian volume, and antral follicle count in women with acne. Gynecol Obstet Invest. 2015; 79:78-82.

9. Sadek IA, Abdul-Mohsen MH. Long-term administration of vitamin A and the process of spermatogenesis. East Mediterr Health J. 1999; 5:123-129.

10. Livera G, Rouiller-Fabre V, Pairault C, et al. Regulation and perturbation of testicular functions by vitamin A. Reproduction. 2002; 124:173-180.

11. Çinar L, Kartal D, Ergin C, et al. The effect of systemic isotretinoin on male fertility. Cutan Ocul Toxicol. 2016; 35:296-299.

12. Amory JK, Ostrowski KA, Gannon JR, et al. Isotretinoin administration improves sperm production in men with infertility from oligoasthenozoospermia: a pilot study. Andrology. 2017; 5:1115-1123.

13. Parsch EM, Ruzicka T, Przybilla B, Schill WB. Andrological investigations in men treated with acitretin (Ro 10-1670). Andrologia. 1990; 22:479-482.

14. Török L, Kádár L, Kása M. Spermatological investigations in patients treated with etretinate and isotretinoin. Andrologia. 1987; 19:629-633.

15. Hui Liu, Jie Li, Li Yu. Effects of acitretin on semen quality and reproductive hormone levels in patients with psoriasis vulgaris. Dermatologica Sinica. 2017; 35:55-58.

16. Onder S, Çırakoğlu A, Nalbant I, et al. Does isotretinoin affect spermatogenesis? a rat study. Egypt J Dermatol Venerol. 2021; 41:75-82.

17. Sengör B, Bayramgürler D, Müezzinoglu B, et al. Effects of acitretin on spermatogenesis of rats. J Eur Acad Dermatol Venereol. 2006; 20:689-692.

18. Cai L, Hales BF, Robaire B. Induction of apoptosis in the germ cells of adult male rats after exposure to cyclophosphamide. Biol Reprod. 1997; 56:1490-1497.

19. Print CG, Loveland KL. Germ cell suicide: new insights into apoptosis during spermatogenesis. Bioessays. 2000; 22:423-430.

20. Lin WW, Lamb DJ, Wheeler TM, et al. In situ end-labeling of human testicular tissue demonstrates increased apoptosis in conditions of abnormal spermatogenesis. Fertil Steril. 1997; 68:1065-1069.

21. Høst E, Lindenberg S, Ernst E, Christensen F. DNA strand breaks in human spermatozoa: a possible factor, to be considered in couples suffering from unexplained infertility. Acta Obstet Gynecol Scand. 1999; 78:622-625.

22. Karadag AS, Ertugrul DT, Tutal E, Akin KO. Isotretinoin influences pituitary hormone levels in acne patients. Acta Derm Venereol. 2011; 91:31-34.

23. Altucci L, Gronemeyer H. The promise of retinoids to fight against cancer. Nat Rev Cancer. 2001; 1:181-193.

24. Gencoglan G, Tosun M. Effects of isotretinoin on spermatogenesis of rats. Cutan Ocul Toxicol. 2011; 30:55-60.

25. Ghasemnezhad R, Mohammadghasemi F, Faghani M, Bahadori MH. Oxytocin can decrease germ cells apoptotic index in testis under acute ischemia reperfusion in a rat model. Iran J Reprod Med. 2015; 13:283-290.

26. Kandirali E, Cayan S, Armagan A, et al. Does the testicular apoptotic index vary with serum gonadotropins and testicular histopathology in infertile men? Urol Int. 2009; 83:349-353.

27. Wing TY, Christensen AK. Morphometric studies on rat seminiferous tubules. Am J Anat. 1982; 165:13-25.

28. Udoumoh AF, Igwebuike UM, Okoye CN, et al. Assessment of age-related morphological changes in the testes of post-hatch light ecotype Nigerian indigenous chicken. Anat Histol Embryol. 2021; 50:459-466.

29. Mohammadnejad D, Abedelahi A, Soleimani-Rad J, et al. Degenerative effect of Cisplatin on testicular germinal epithelium. Adv Pharm Bull. 2012; 2:173-177.

30. Abou-haila A, Tulsiani DR. Signal transduction pathways that regulate sperm capacitation and the acrosome reaction. Arch Biochem Biophys. 2009; 485:72-81.

31. Visconti PE, Westbrook VA, Chertihin O, et al. Novel signaling pathways involved in sperm acquisition of fertilizing capacity. J Reprod Immunol. 2002; 53:133-150.

32. Pelit ES, Katı B, Akın Y, Yeni E. The impact of environmental stress factors on sperm. Androl Bul. 2017; 19:61-64.

33. Nasr M, Abd-Allah H, Ahmed-Farid OAH, et al. A comparative study between curcumin and curcumin nanoemulsion on high-fat, high-fructose diet-induced impaired spermatogenesis in rats. J Pharm Pharmacol. 2022; 74:268-281.

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