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Histological studies on the effect of soyabeans, as a main ingredient in fast food, on fertility of pubescent male mice

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Abstract

Soybeans, a widely consumed legume, contain phytoestrogens that mimic estrogen and may influence reproductive health. With the increasing prevalence of soy-based ingredients in fast food, concerns have been raised regarding their potential endocrine-disrupting effects, particularly in males during puberty. This study investigates the histological impact of a soy-rich diet on the testes of pubescent male mice, providing insights into its effects on spermatogenesis and testicular morphology.

Eighteen male albino mice were divided into three groups: a control group fed a standard diet and two experimental groups fed diets containing 50% and 70% soy protein, respectively, for six weeks. Testicular tissue was collected and analyzed using histological techniques to assess structural and cellular changes. Results demonstrated significant alterations in the testes of soy-fed mice, with higher soy intake correlating with increased disruptions. The 50% soy diet resulted in moderate testicular atrophy, reduced spermatogenesis, and vacuolization of spermatogenic and Sertoli cells. The 70% soy diet caused severe degeneration, characterized by extensive germ cell apoptosis, seminiferous tubule disorganization, and conspicuous alterations of interstitial tissue . Furthermore, increased vascular congestion and immune cell infiltration suggested an inflammatory response, possibly induced by oxidative stress.

These findings align with previous research indicating that phytoestrogens can interfere with male reproductive development. The observed testicular damage suggests that prolonged exposure to soy-based diets, particularly in fast food, may have adverse effects on male fertility. Given the global rise in fast-food consumption, further studies are needed to determine long-term reproductive consequences and to assess potential reversibility of these effects. This study highlights the importance of dietary awareness and the need for updated nutritional guidelines to mitigate possible risks associated with excessive soy intake.

Key words: soya beans – testis – male mice- fertility

1.Introduction

Soybeans are a super legume for many reasons and overall wellbeing. High in protein and a great plant based alternative to animal protein so good for vegetarians and vegans (Messina, 2016). Soy contains isoflavones which have been shown to reduce heart disease, osteoporosis and hormone related cancers (Setchell et al., 1999). Soy also supports metabolic health and weight management by increasing satiety and reducing overall calorie intake (Rebello et al., 2014). So soybeans are a great addition to your diet and long term health.

Soybeans have been studied for their effects on various organs and the research shows both benefits and concerns. The heart benefits from soy consumption as soy protein and isoflavones lower LDL cholesterol and improve cardiovascular health (Anderson et al., 1995). In the liver soy isoflavones regulate lipid metabolism and may protect against fatty liver disease by reducing triglyceride accumulation (Liu et al., 2010). Soy consumption has been shown to improve kidney function as soy protein puts less burden on renal function than animal protein making it good for people with kidney disease (Soroka et al., 2012). In the endocrine system soy isoflavones interact with estrogen receptors and affect hormone related organs like the breast and prostate; some studies show protective effects against hormone related cancers while others show complex hormonal effects (Messina, 2016). Soy has also been shown to benefit bone health by increasing bone mineral density and reducing osteoporosis risk especially in postmenopausal women (Arjmandi et al., 2003). These findings show the different effects of soybeans on various organs and confirm its role in health and disease prevention.

The greater incidence of fast food in diets nowadays is raising serious health concerns as far as reproductive health is concerned. Fast food items are mostly heavy on meat and soybeans, which are primarily used as fillers, meat substitutes, and emulsifiers among other processed ingredients in prepared products. Soybeans tend to have high phytoestrogen concentration in the form of isoflavones, which has been extensively researched for its estrogenic effects on mammals (Messina, 2016).
Structurally, these compounds mimic endogenous estrogen and can bind to the estrogen receptors, thus making it plausible to alter the hormonal balance and affect reproductive function (Cederroth et al., 2010). Hence, it is important to look at the histological effects of fast foods domestically grown with soybeans on reproductive tissues, thereby increasing the significance of understanding these products with such high consumption.

Male fertility is a complex physiological process that requires the proper functioning of the hypothalamic-pituitary-gonadal (HPG) axis, normal spermatogenesis, and the integrity of testicular structures (Sharpe, 2010). As in humans, during puberty, the male mice undergo significant hormonal changes really affecting their sexual maturation and hence become an ideal model for studying the effects of diet on reproductive development. Past research states that phytoestrogens can affect testicular morphology, sperm production, and overall reproductive capacity (Wisniewski et al., 2003). However, it remains an inquiry field discovering what changes these diets cause linguistically histologically, especially when consumed as fast food.

Indeed, fast food has become a vital element of modern dietary habits, not only among adolescents but also among young adults. Beyond that, most of these fast food products are sweet enabled with soy derivatives such as soy protein isolates, soy lecithin, or textured vegetable proteins (TVP) to serve as cost-effective substitutes for animal-based ingredients (Krebs-Smith et al., 2010). Therefore, regular consumers of fast food are greatly inundated with high levels of phytoestrogens while completely unaware of their potential biological effects.

Particular concern among those fast food diets with soy diets is possible cumulative exposure to isoflavones, which may in turn affect reproductive health over time. Consistent with this notion is research indicating that moderate phytoestrogen consumption during crucial periods in development, such as puberty, could carry long-term reproductive consequences (McClain et al., 2007). Hence, histological evaluation of soybased fast-food diet feeding in pubescent male mice may reveal critical information regarding the effect of such diet on reproductive health.

This study aims to expand existing knowledge through a detailed histological examination of the testes in pubescent male mice exposed to a soy-rich fast-food diet

Given the nutritional and public health implications, these findings may have widespread relevance, particularly as fast-food consumption continues to rise globally (Reed et al., 2020). If significant histological changes in testicular structure are observed due to soy-rich diets, this could underscore the need for updated nutritional guidelines and increased consumer awareness regarding phytoestrogens (Messina, 2016). Understanding the potential endocrine-disrupting effects of soy-based foods may also contribute to broader discussions on food safety and reproductive health policy.

2.Materials and Methods

Experimental animals

Eighteen male developing albino mice (6-weeks old) with body weights ranging from 16-20 g were obtained from National Research Centre (NRC),Dokki, Egypt. The animals were housed in clear plastic cages (3 animals/cage) with wood chips as bedding in a room with controlled conditions (temperature range of 25 ± 2 °C, relative humidity of 55 ± 5 % and a 12 h light/dark cycle). The animals were fed *ad libitum* with a standard diet and acclimatized in the laboratory for one week (from postnatal day 42 to postnatal day 49) prior to experimentation. Following the sci.1332502002 permission code, the Ain Shams University Ethical Agreement Committee made sure that every animal research followed their normal guidelines for the care and use of experimental animals.

Experimental design

The mice were divided into three groups (6 animals each) as follows:
Group C: the control group was kept in an identical situation to the experimental groups but did not receive a diet containing soy within the period of experiment.
Group A: in this group 6 mice received a diet with 50% soy protein (Modaresi et al. 2011)
Group B: in this group 6 mice received a diet with 70% soy protein.
Since the base of the experiment was feeding the mice with soy protein as food, the amount of food eaten by the animals during a day was calculated for a week through calculating the difference in weight of the given food. The food used for feeding the mice was ground into powder using electric mixer and then was mixed with 50 and 70% soy based on the dose required for each experimental group. The food was then made again in the form of paste and was made available to be used by animals. The experiment lasted 6 weeks for each animal.

Collection of tissue samples

After 42 days (6 weeks) of feeding, animals were scarified by fast decapitation. testicular tissue samples were taken from mice of all groups for histological study.

Histological preparations

For the histological and histopathological studies of the target organ, small pieces of the testis of male mice of control and experimental animals were removed and fixed instantaneously in aqueous Bouin fixative. The fixation was achieved 24 hours. Then the specimens were dehydrated in ascending series of ethyl alcohol, cleared in terpineol for 2-3 days and washed in benzene. The specimens were placed in 3 changes of melting paraffin wax for infiltration and embedded in paraffin blocks. Serial transverse sections of about 5 µm thick were prepared and mounted on clean glass slides. Paraffin sections were stained with hematoxylin and eosin (Bancroft and Stevens 2016), dehydrated, cleared in xylol and mounted by DPX.

Stained sections of testis of control and experimental groups were examined carefully; and photomicrographs were made as requested.

The average body weight of male mice of the control and experimental groups were recorded at the beginning of every week and for this statistical study, we consulted SPSS, Chicago, IL,Statistical Package for the Social Sciences, Version 23.1 for Windows.

3.Results

Mean body weight

The control and treated mice's mean bo dy weights were noted in the current in vestigation (Table 1 and Fig. 1).
It is evident from the data in table (1) t hat the mean body weight of the treate d and control animals varied significan tly.
The mean body weight of the treated m ice was significantly higher than that of the control mice after six weeks of trea tment ($P < 0.001$).
Additionally, animals in group B exhib it the greatest gain when compared to t hose in group A.

Table (1): Illustrating the effect of feeding with soyabeans on the average body weight (g) of pubescent male mice.

Weeks	Groups	Number of animals	Mean body weight	Std. Deviation	Std. Error	Sig.
week 1	C	6	16.267	.3882	.1585	
	A	6	20.767	1.4569	.5948	***
	B	6	18.533	1.2956	.5289	***
week 2	C	6	17.333	1.1183	.4566	
	A	6	22.183	1.5955	.6514	***
	B	6	21.617	1.4233	.5810	***
week 3	C	6	20.183	1.4077	.5747	
	A	6	26.967	2.1078	.8605	***
	B	6	23.950	1.8929	.7728	***
week 4	C	6	21.033	2.0216	.8253	
	A	6	27.417	2.0595	.8408	***
	B	6	25.667	1.4024	.5725	***
week 5	C	6	22.300	1.3550	.5532	
	A	6	27.617	1.7837	.7282	***
	B	6	27.867	1.4250	.5818	***
Week 6	C	6	22.967	1.0857	.4432	
	A	6	28.550	1.6956	.6922	***
	B	6	29.300	1.0354	.4227	***
Week 7	C	6	23.217	.6616	.2701	
	A	6	30.333	1.4610	.5965	***
	B	6	30.900	1.1507	.4698	***

Group C: control group , group A mice received a diet with 50% soy protein, group B: mice received a diet with 70% soy protein

P>0.05 =not significant, P≤0.05 =significant (*), P<0.01 =high significant (**), P<0.001 or 0.005 = highly significant (***)

Fig. (1): Showing the relationship between soybean the dietary periods and mean body weight.

Histological and Histopathological Observations

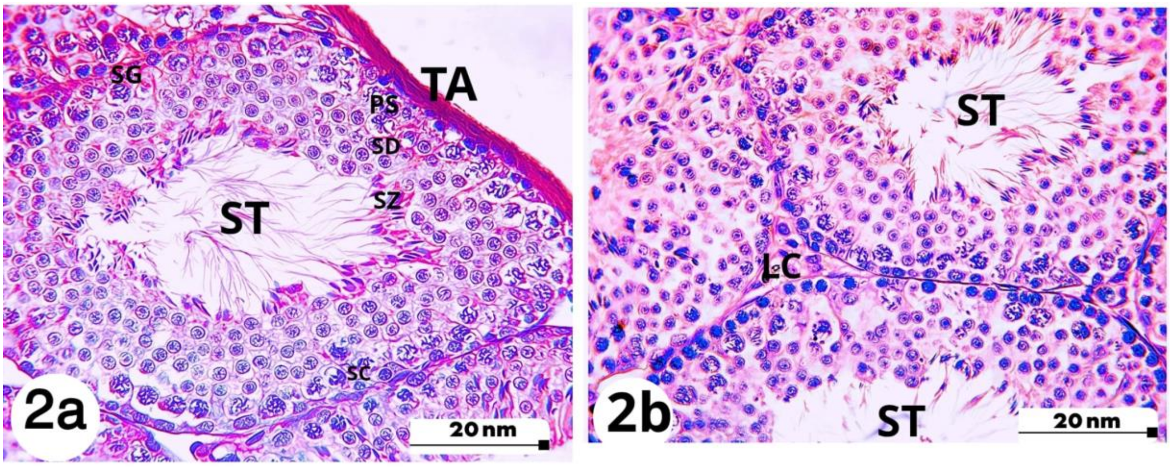

Fig. 2

Fig.2 a& b: Photomicrographs of testes sections of control mice **a:** Showing the tunica albuginea (TA), seminiferous tubules (ST) including successive stages of spermatogenesis, spermatogonia (SG), primary spermatocytes (PS), forms of spermatids (SD) and spermatozoa (SZ) surrounding a central lumen. the basal lamina is where Sertoli cells (SC) are connected by their bases**. b:** illustrating seminiferous tubules (ST) and also Leydig cells (LC).

I- Control mice's testes:

The transverse section of a control mouse testis under a microscope reveals the typical structure of seminiferous tubules, Seminiferous tubules appear as circular or oval structures densely packed within the testis. They are surrounded by a thin basement membrane composed of connective tissue. Seminiferous tubules are tightly packed and surrounded by interstitial tissue. The seminiferous tubules are lined with germinal epithelium, consisting of spermatogenic cells at different stages of development, including spermatogonia, near the basement membrane, round cells with dark nuclei, Primary spermatocytes which are larger cells, undergoing meiosis. Spermatids the small, round cells closer to the lumen, which transform into spermatozoa. Spermatozoa the mature sperm cells with elongated heads, present in the lumen). And Sertoli cells, these elongated, pale-staining cells which provide structural and nutritional support, are also present. (Fig 2a&b.)

The interstitial spaces between the seminiferous tubules contain Leydig cells, responsible for testosterone production. The tubules display an organized arrangement with round or oval-shaped profiles, and the lumen often contains spermatozoa. Staining typically highlights cell nuclei in darker hues (purple/blue with hematoxylin and eosin stain), while the cytoplasm and extracellular components appear lighter (pink/yellow). The interstitial area also contains blood vessels, and connective tissue to support testicular function (Fig. 2b).

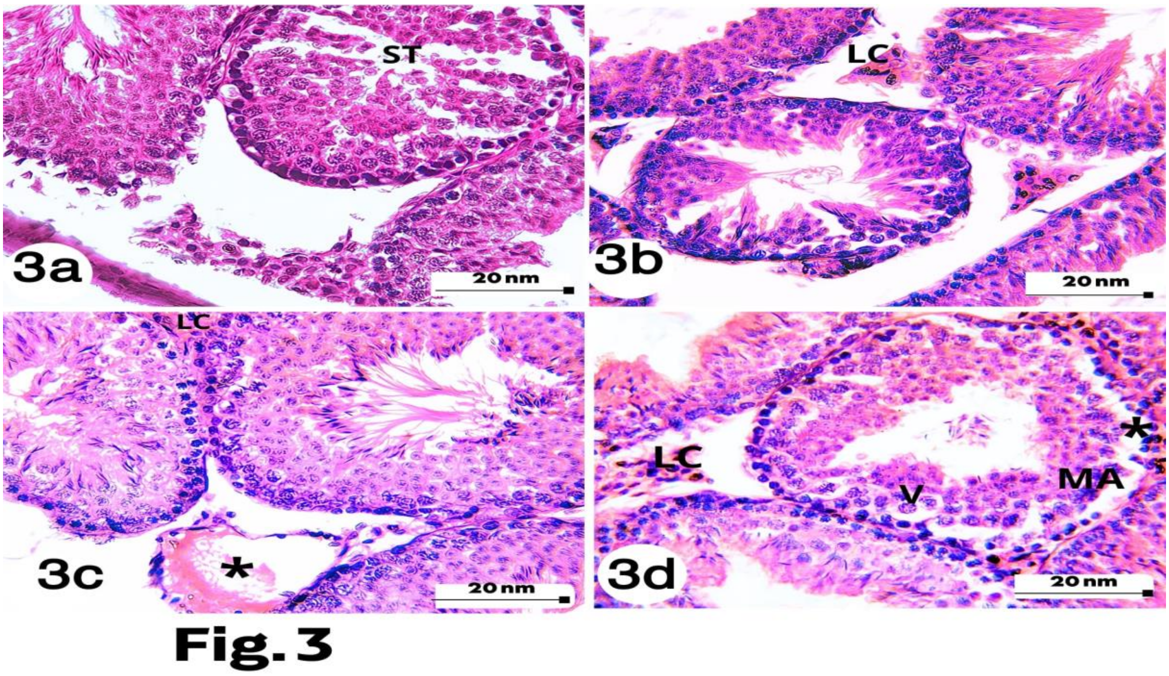

Fig.3 a, b. c& d: Photomicrographs of testes sections of mice received a diet with 50% soy protein exhibiting a: degenerative changes of some seminiferous tubules (ST). b: Obvious wide intertubular spaces containing interstitial tissues with atrophied Leydig cells (LC) C: Hypoplasia of Leydig cells (LC) and congested blood vessel (*). d: seminiferous tubules showing exfoliation (*), maturation arrest (MA) with different vacuoles (V) and hypoplasia of Leydig cells (LC).

II- Group A mice received a diet with 50% soy protein

The normal rounded and compact arrangement of seminiferous tubules appears disorganized. Some tubules exhibit irregular shapes, shrinking, or deformation. Tubular atrophy is evident in some sections, with reduced epithelial height (Fig. 3b). The seminiferous epithelium shows depletion of spermatogenic cells. Also, reduction in spermatogenesis appears as a noticeable decline in spermatozoa (Fig. 3 b & d) within the lumen of seminiferous tubules and some tubules appear empty or with sparse spermatozoa, indicating impaired spermatogenesis (Fig.3b&d).

Interstitial tissue showed alterations, expansion of interstitial spaces between tubules, potentially due to edema. With signs of Leydig cell degeneration, which may affect testosterone production. The Blood vessels in the interstitial tissue show congestion, in addition to the presence of infiltrating immune cells suggests an inflammatory reaction possibly linked to oxidative stress or inflammatory responses. (Fig.3 a,c &d)

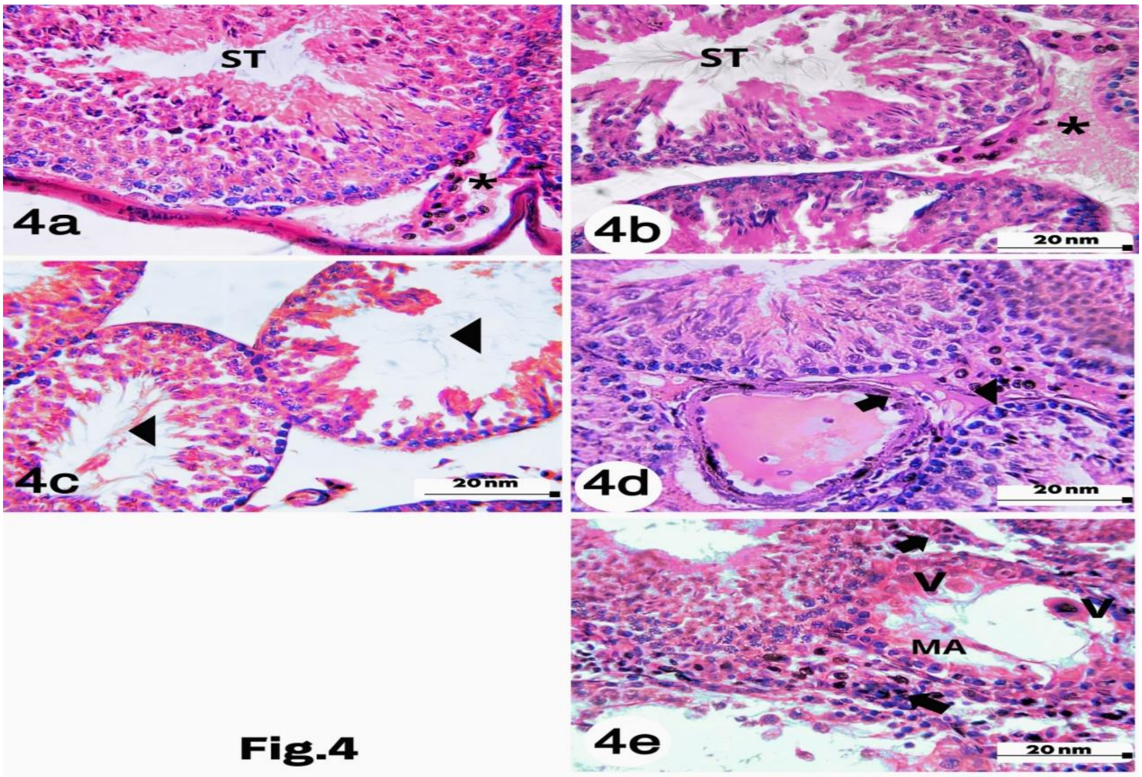

Fig.4 a, b, c, d& e: Photomicrographs of testes sections of mice received a diet with 70% soy protein illustrating a&b: severe degenerative changes of seminiferous tubule (ST) lost their normal configuration and hemorrhagic edema (*) is also seen.c: complete absence of spermatogonial series in the lumen of the most seminiferous tubules (arrowhead) with azoospermia in most tubules. d: congested and thickening of blood vessel (arrow) and atrophy of interstitial cells (arrowheads). E: sever degeneration of seminiferous tubules and maturation arrest (MA) with different vacuoles (V) and interstitial tissue invaded by inflammatory cells (arrows).

III- Group B mice received a diet with 70% soy protein

The testes reduced in size and show pale discoloration, indicating possible atrophy. Surface irregularities or shrinkage may be observed due to loss of normal tissue integrity. Possible softening of the testes due to degenerative changes. Seminiferous tubules exhibit, irregular shape, and loss of cellular organization. with reduction or absence of various spermatogenic layers (spermatogonia, spermatocytes, and spermatids) in most tubules. And increased number of apoptotic cells, leading to impaired sperm production.

Cytoplasmic vacuoles appear within the spermatogenic and Sertoli cells, indicating metabolic disturbances as seen in figure 4 b& c.

Marked interstitial edema and widened interstitial spaces, suggesting chronic damage. Leydig cell depletion or structural abnormalities, potentially leading to decreased testosterone production. the vascular alterations exhibits in the form of congestion and hemorrhage as seen in figure 4 d & e which showed dilated blood vessels with focal hemorrhagic areas, indicating vascular compromise with the presence of mononuclear cell infiltration in some areas, suggesting an immune response to tissue damage.

4.Discussion

The present study provides a detailed histological analysis of the effects of a soy-rich diet on the testicular structure of pubescent male mice. Our findings demonstrate that increased soy protein consumption significantly impacts body weight, testicular morphology, spermatogenesis, and interstitial tissue integrity, consistent with previous studies investigating phytoestrogen effects on male reproductive health.

Phytoestrogens, particularly isoflavones such as genistein and daidzein, are structurally similar to endogenous estrogens and have been shown to exert both agonistic and antagonistic effects on estrogen receptors (Setchell & Cassidy, 1999). These compounds have been previously linked to alterations in testosterone levels, sperm quality, and testicular histology in both human and animal studies (Atanassova et al., 2000). Our results align with these findings demonstrating a dose-dependent effect of soy protein intake on testicular histoarchitecture, where higher soy consumption resulted in greater disruption of seminiferous tubule organization, germ cell apoptosis, and interstitial tissue inflammation.

Histological examination revealed that mice fed a 50% soy protein diet exhibited significant increase in body weight and mild testicular atrophy, reduced spermatogenic cell populations, and occasional vacuolization in spermatogenic and Sertoli cells. These findings are consistent with those of Adachi et al. (2004), who reported decreased seminiferous tubule diameter and increased germ cell apoptosis in rodents exposed to high doses of phytoestrogens. In the 70% soy protein group, highly significant increase in body weight and testicular damage were more pronounced, with significant tubular atrophy, germ cell exfoliation, and reduced Leydig cell populations. These results corroborate earlier studies by Nagao et al. (2001), who suggested that prolonged exposure to phytoestrogens can interfere with testicular development and function.

The observed vascular alterations in the present investigation, including congestion and hemorrhagic regions, further suggest that a soy-rich diet may disrupt normal testicular microcirculation, potentially leading to hypoxia and oxidative stress. Similar findings have been reported by Zhang et al. (2019), who emphasized the role of phytosterols in inducing testicular oxidative damage. The presence of immune cell infiltration in the interstitial spaces also supports the hypothesis that phytoestrogen exposure may trigger inflammatory responses, contributing to impaired spermatogenesis and testosterone synthesis (Zhou et al., 2021).

Furthermore, certain dietary supplements and food additives have also been linked to testicular histological changes. High doses of vitamin A, while essential for spermatogenesis, have been reported to induce oxidative stress and apoptosis in testicular tissue when consumed excessively (Huang et al., 2018). Similarly, excessive intake of vitamin C and E supplements, commonly used for their antioxidant properties, may paradoxically promote oxidative damage in the testes, affecting sperm viability and morphology (Khalaf et al., 2019).

Testosterone and anabolic steroid supplementation, frequently used for performance enhancement, have been extensively studied for their adverse effects on male fertility. Chronic use of exogenous testosterone can suppress endogenous gonadotropin secretion, leading to testicular shrinkage, impaired spermatogenesis, and reduced testosterone synthesis (Coviello et al., 2005). Additionally, synthetic estrogens, often used in hormonal treatments, have been shown to disrupt the hypothalamic-pituitary-gonadal axis, causing testicular atrophy and fertility impairment (Sharpe, 2010).

Despite the compelling histological evidence, some studies have suggested that the effects of phytoestrogens on male reproductive health remain controversial. Epidemiological studies in human populations have yielded mixed results, with some suggesting no significant impact on fertility parameters, while others report reduced sperm counts and altered hormonal profiles (Reed et al., 2020). These discrepancies may be attributed to differences in exposure levels, duration of intake, genetic predisposition, and the presence of other dietary components.

Our findings underscore the potential risks associated with excessive soy consumption, particularly in adolescent males during critical developmental periods. Given the rising prevalence of fast-food diets enriched with soy-based derivatives, these results highlight the need for further investigation into the long-term reproductive consequences of phytoestrogen exposure. Future studies should aim to elucidate the precise molecular mechanisms underlying these histological changes and assess whether these effects are reversible upon the cessation of soy intake.

5. Interpretation of Results

Table (2): Illustrating comparison of testicular changes among groups

Feature	Control Group (Normal Diet)	50% Soybean Diet	70% Soybean Diet
Gross Morphology	Normal-sized testes, pinkish and firm	Slightly reduced size, mild pallor	Significantly smaller, atrophic testes, pale & soft
Seminiferous Tubules	Well-organized, full spermatogenic layers, normal diameter	Some tubules show mild atrophy & irregular shape	Severe atrophy, disorganized tubules, loss of spermatogenesis
Sertoli Cells	Normal morphology	Mild vacuolization, slightly reduced support function	Extensive vacuolization, severe structural damage
Spermatogenesis	Complete, all germ cells present	Reduced spermatogenesis, some germ cell loss	Severe impairment, germ cell apoptosis, empty tubules
Germ Cell Detachment	None	Occasional exfoliation of immatur cells	High degree of sloughing, lumen filled with

			immature germ cells
Leydig Cells	Normal distribution, functional testosterone production	Mild reduction, slightly disorganized	Severe reduction, impaired testosterone synthesis
Interstitial Tissue	Normal, minimal fibrosis	Mild interstitial edema	Severe interstitial edema
Basement Membrane	Normal, intact	Slightly thickened in some tubules	Thickened & hyalinized, indicating severe damage
Blood Vessels & Inflammation	Normal, no congestion or hemorrhage	Mild congestion, occasional inflammatory cells	Significant congestion, vascular damage, immune cell infiltration
This may be return to		• The effect on estrogen receptors. • Disruption of testicular microcirculation. • Increase the hypoxia and oxidative stress.	

6.conclusion

This study contributes to the growing body of evidence linking dietary phytoestrogens to testicular histopathology. While moderate soy consumption may offer health benefits, excessive intake could pose risks to male reproductive health, particularly during puberty. These findings reinforce the importance of dietary awareness and the potential need for revised nutritional guidelines to mitigate unintended endocrine-disrupting effects of soy-rich diets.

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دراسات نسيجية على تأثير فول الصويا كمكون رئيسي في الوجبات السريعة على خصوبة ذكور الفئران قبل البلوغ.

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قسم العلوم البيولوجية و الجيولوجية –كلية التربية – جامعة عين شمس

الفرقه الرابعه بيولوجي انجليزي 2024–2025

فول الصويا، وهو من البقوليات واسعة الاستهلاك، يحتوي على مركبات الفيتواستروجين التي تُحاكي تأثير هرمون الإستروجين وقد تؤثر على الصحة الإنجابية. ومع ازدياد انتشار مكونات الصويا في الوجبات السريعة، أثيرت مخاوف بشأن آثارها المحتملة على الغدد الصماء، لا سيما عند الذكور خلال فترة البلوغ. تهدف هذه الدراسة إلى استقصاء التأثيرات النسيجية لنظام غذائي غني بالصويا على خصى ذكور الفئران في مرحلة البلوغ، من خلال تحليل تأثيره على تكوّن الحيوانات المنوية وبنية الخصية.

تم تقسيم ثمانية عشر فأرًا أبيض ذكرًا إلى ثلاث مجموعات: مجموعة ضابطة تغذت على نظام غذائي قياسي، ومجموعتين تجريبيتين تغذتا على أنظمة غذائية تحتوي على 50٪ و70٪ من بروتين الصويا، على التوالي، لمدة ستة أسابيع. تم جمع أنسجة الخصية وتحليلها باستخدام تقنيات نسيجية لتقييم التغيرات البنيوية والخلوية.

أظهرت النتائج تغيّرات ملحوظة في خصى الفئران التي تغذت على الصويا، حيث ارتبطت زيادة تناول الصويا بازدياد الاضطرابات. أدى النظام الغذائي الذي يحتوي على 50٪ صويا إلى ضمور متوسط في الخصية، وانخفاض في تكوّن الحيوانات المنوية، وتكوّن فجوات فى الخلايا المنوية و خلايا سيرتولي. أما النظام الغذائي الذي يحتوي على 70٪ صويا فقد تسبب في تدهور شديد، تميز بزيادة واضحة في موت الخلايا الجرثومية، واضطراب في الانبيبات المنوية، وتغيرات بارزة في النسيج البيني. إضافةً إلى ذلك، لوحظ احتقان وعائي متزايد وتسلل خلايا التهابية، مما يشير إلى وجود استجابة التهابية يُحتمل أن تكون ناجمة عن الإجهاد التأكسدي.

وتشير الأضرار الملحوظة في الخصية إلى أن التعرض الطويل للأنظمة الغذائية المعتمدة على الصويا، خصوصًا في الوجبات السريعة، قد يكون له آثار سلبية على خصوبة الذكور. وبالنظر إلى الارتفاع العالمي في استهلاك الوجبات السريعة، هناك حاجة لمزيد من الدراسات لتحديد العواقب التناسلية طويلة الأمد وتقييم إمكانية عكس هذه الآثار. تُبرز هذه الدراسة أهمية التوعية الغذائية وضرورة تحديث الإرشادات التغذوية للحد من المخاطر المحتملة المرتبطة بالاستهلاك المفرط للصويا.

الكلمات المفتاحية :فول الصويا – الخصية – فئران ذكور – الخصوبه

Ethical Approval Certificate

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Research Title : 《Histological studies on the effect of soybeans, as a main ingredient in fast food, on fertility of pubescent male mice》

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Upon completion of the revision of information provided by applicant

This study has been approved by the Research Ethics Commitee

Ethics Approval

2025-02-26

Head of Research Ethics Committe	Vice Dean for Graduate Studies and Research Affairs	Dean
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