Iranian Journal of Toxicology
ARAK UNIVERSITY OF MEDICAL SCIENCES

January 2024, Volume 18, Number 1

Research Paper

Evaluating the Anti-fertility Potentials of 3-Monochloropropane-1, 2-diol (Alpha-Chlorohydrin) and Testosterone in Adult Male Wild Nile Grass Rats (*Arvicanthis niloticus*) for Rodent Control

Zeinab Hassan [1,*], Mahmoud Ashry [2], Magdy Wilson [1], Mohsen A. Moustafa [2], Ayat Taha [3]

[1] *Agriculture Research Center, Doki, Egypt*
[2] *Zoology Department, Faculty of Science, Al-Azhar University, Assuit, Egypt*
[3] *Zoology Department, Faculty of Science, Ain-shams University, Cairo, Egypt*

Scan to access website

How to cite this paper:
Hassan Z, Ashry M, Wilson M, A. Moustafa M, Taha A. Evaluating the Anti-fertility Potentials of 3-Monochloropropane-1, 2-diol (Alpha-Chlorohydrin) and Testosterone in Adult Male Wild Nile Grass Rats (Arvicanthis niloticus) for Rodent Control. Iranian Journal of Toxicology. 2024; 18(1):29-38. **doi:** 10.61186/IJT.18.1.29

10.61186/IJT.18.1.29

Article info
Received: 18/08/2023
Accepted: 02/11/2023
Published: 28/01/2024

*** Corresponding author:**
Mahmoud Ashry, PhD, Faculty of Science, Al-Azhar University, 71554 Assiut, Egypt. E-mail: mahmoud_ashry20@yahoo.com

ABSTRACT

Background: The Nile grass rat (*Arvicanthis niloticus*) is the most serious vertebrate pest in Egypt, causing significant economic losses to cultivated crops and stored foodstuffs. The purpose of this study was to evaluate the potential anti-fertility effects of alpha-chlorohydrin (ACH) and exogenous testosterone on this pest.

Methods: Rats were orally administered ACH at a dose of 70 mg/kg for 5 days and exogenous testosterone at 25 mg/kg three times a week for 3 weeks. The anti-fertility effects of both agents were assessed after 24 hours of exposure.

Results: The findings revealed that both ACH and exogenous testosterone significantly reduced the serum ATPase, Esterase, and G3PDH activities, hormonal fertility, testosterone levels, and LH and FSH levels. Also, the agents caused slight increases in ASAT, ALAT, GGT, ALP activities, urea, uric acid, and creatinine levels. There was a noticeable decline in the oxidative functions of the testis and epididymis; with CAT, SOD, and GSH levels in these organs being dramatically inhibited, while the levels of MDA and NO increased. Further, both agents led to a decrease in the weight of the reproductive organs, sperm count and motility, and induced histological changes in the epididymis and testis. Moreover, there was a reduction in the expression of immunohistochemical markers of androgen receptor proteins and Wilms' tumor nuclear protein-1 in both testicular and epididymal tissues.

Conclusion: The results indicate that ACH and testosterone induce infertility in male Nile rats. Therefore, the use of ACH and testosterone is recommended for integrated rodent control and management.

Keywords: Alpha-Chlorohydrin; Anti-fertility; *Arvicanthis niloticus*; Pest control; Testosterone
Abbreviations: PND = Postnatal days (from PND-4 to PND-90).

Introduction

Previous toxic methods used to control rodent pests have proven ineffective and inhumane because mortality control has a short-term effect on the rodent populations. Rodents reproduce rapidly and the terminated ones are replaced soon. Also, traditional methods have toxic effects on non-target animals and cause secondary environmental pollution [1]. The use of male anti-fertility agents for rodent control has gained attention due to the potential efficiency in disrupting male spermatogenesis [2-4].

Alpha-chlorohydrin (ACH), can cross the blood-brain and blood-testis barriers. ACH, often referred to as 3-monochloropropane-1, 2-diol, or 3-chloro-1,2-propanediol, is a well-recognized contaminant that has been detected in several types of foods [2, 3] and is often found in tap water [3]. This agent has proven to be species-specific, impairing the fertility of animals such as rats [5], hamsters [6], and the Egyptian fruit bat *Rousettus aegyptiacus* [2, 3]. However, ACH does not affect the fertility of three rodent species: Shaw's jird, *Meriones shawi*, the lesser Egyptian gerbil, *Gerbillus gerbillus*, and the Cairo spiny mouse, *Acomys cahirinus* [7].

Testosterone is a vital male reproductive hormone that controls several processes, such as sex differentiation, spermatogenesis, male sex, and fertility [8]. It is responsible for several processes in

the development of sexual characteristics, such as testicular the descent, spermatogenesis, testes and penis enlargement, and enhanced libido [9].

Spermatogenesis is only possible in Sertoli cells, which interact directly with the germ cells. Thus, dysfunction of these cells often results in spermatogenic failure [10]. Various cells exhibit distinct markers that are often indicative of their cell types. The identification of Sertoli cells involves the use of certain markers, such as Wilms' tumor (WT-1) and androgen receptor (AR), which exhibit higher levels of expression in Sertoli cells [11, 12].

Considering the above facts, we planned to evaluate the anti-fertility potential of ACH and testosterone in adult male wild Nile grass rats (*Arvicanthis niloticus*), and their effectiveness as anti-fertility agents in rodent pest management.

Materials and Methods

Chemicals

Alpha-chlorohydrin (ACH) and exogenous synthetic testosterone were supplied by Sigma Aldrich (St. Louis, MO, USA). All other chemicals were purchased from Pharmaceutical Chemicals Co Ltd (Assuit, Egypt).

Animals

Forty-five adult male Nile grass rats weighing approximately 124 ± 0.87 g were used in this study. The rats were obtained from the Al-Azhar farm, affiliated with the Faculty of Agriculture, Al-Azhar University. Prior to conducting the experiments, the rats were housed in separate cages in the animal house of the Zoology Department, Faculty of Science, Al-Azhar University, under normal 12-hour light/dark cycle. The rats had free access to standard laboratory diet and water, *ad libitum.*

Animal Study Design

The rats were randomly divided into three groups of 15 each. The 1stgroup (controls) received distilled water. The 2ndgroup received ACH at 70 mg/kg for 5 days via oral gavage [13]. The 3rdgroup received testosterone (25 mg/kg, 3 weeks, 3 times/week) via oral gavage, following the method described by Callies, *et al*. [14], with minimal modifications. Fresh preparations were diluted in distilled water immediately before use. Clinical signs were recorded in rats throughout the experimental period.

Blood and Tissue Sampling

Each animal was weighed after treatment, fasted overnight, and then weighed again. After anesthesia (inhalation of diethyl ether), blood samples were withdrawn from the retro-orbital plexus, using heparinized and sterile glass capillaries. The sera were divided, stored at -80°C, and centrifuged at 3000 rpm for 10 min, until the biochemical analyses. The animals were sacrificed shortly after blood collection and their testes and epididymides were dissected. Portions of the testes and cauda epididymides were washed in normal saline, dried, and wrapped in aluminum foils for further biochemical analysis Another portion of the cauda epididymis was placed in saline (0.9%) to further examine sperm characteristics. Other portions of the epididymis and testes were soaked in 10% neutral formalin for later histopathological and microscopic examinations. At necropsy, the testes, epididymides, seminal vesicles, and prostate gland were cleaned for the adhering fat, excised, and weighed. The index weight of reproductive organs was calculated using the formula: [(organ weight /body weight) × 100].

Biochemical Determinations

A Shimadzu spectrophotometer (UV-vis 1201, Japan) was used for all biochemical measurements and readings. The serum aminotransferases (ALAT & ASAT) levels were measure using the reagent kits obtained from the German company, Human Gesell Schaft für Biochemical und Diagnostic GmbH. The kinetic method for measuring serum γ-glutamyl transferase activity was followed based on Schumann and Klauke [15, 16], using the reagent kits obtained from Bio Systems S.A. Costa Brava 30, Barcelona, Spain. Also, the serum alkaline phosphatase activity (ALP) was calculated based on DiaSys reagent kits obtained from DiaSys Diagnostic Systems GmbH, Germany, as described earlier by Moss and Henderson [17]. Another kit was purchased from Biodiagnostic, Dokki, Giza, Egypt, and used to measure the serum levels of urea, uric acid, creatinine, as each of these agent has been described by Chaney *et al*. [18], Husdan and Rupoport [19], and Trivedi, *et al*. [20], respectively.

Oxidative Stress Markers of Testis & Epididymis

The testis and epididymis malondialdehyde (MDA), nitric oxide (NO), and reduced glutathione (GSH) levels, as well as superoxide dismutase (SOD) and catalase (CAT) activities were determined using ELISA (Dynatech microplate reader; model MR-5000, ON, Canada). The ELISA kits were purchased from SinoGeneClon Biotech Co., Hang Zhou, China.

Determination of Testosterone, LH, FSH, ATPase, Esterase, and G3PDH

Similarly, we used the same Dynatech microplate reader to measure the luteinizing hormone (LH), serum testosterone, follicular stimulating hormone (FSH), ATPase, Esterase, and G3PDH concentrations, based on the rat ELISA kits obtained from SinoGeneClon Biotech

Sperm Count and Motility

The cauda epididymis tissues were removed and incubated for 15 minutes at 38°C in 2ml normal saline. A hemocytometer was used under a high-power light microscope to assess the motility and count the sperms, expressed as 10^6/ml.

Histopathology

The paraffin sections at 5µm thick were stained with eosin and hematoxylin [3, 4] and examined under light microscopy.

Morphometric Analyses

Seminiferous tubule diameter, epithelial lining and tunica albuginea thicknesses in the testis were measured under light microscopy. Additionally, the epididymal ductus diameter and height were similarly examined. For each set of samples, ten non-overlapping photomicrographs were taken at 400x magnification.

Johnsen's Score for Spermatogenesis

Spermatogenesis was evaluated using Johnsen's testicular scoring system [21], providing a semi-quantitative assessment. Each sectioned tubule was rated from "10" to "1", based on the presence or absence of the primary cell types as presented in Table 1. The implied rating was determined by randomly selecting 10 seminiferous tubules per rat.

Table 1. Classification of Johns scores of spermatogenesis

Scores	Definitions
10	Complete spermatogenesis
9	Presence of spermatozoa with random spermatogenesis
8	Presence of few spermatozoa
7	Presence of many spermatids
6	Presence of few spermatids
5	No spermatozoa or spermatids but many spermatocytes present
4	Presence of few spermatocytes
3	Presence of spermatogonia
2	Presence of Sertoli cells and no germ cells
1	Almost empty lumens

Immunohistochemistry Examinations

The paraffin-embedded tissue slices were rehydrated using ethanol. Then these sections were treated (15 min) with 10mM citrate buffer (pH 6) in the case of androgen receptor (AR) staining, or with 0.001 M EDTA buffer (15 min) at 95ºC for Wilms' tumor nuclear protein (Wt-1) staining. The sections were incubated overnight at 4ºC, followed by immersion in 1% hydrogen peroxide for 10 minutes. These samples were then immersed in 1% bovine serum albumin (BSA) for 30 minutes. Various primary antibodies were then applied to these sections. This investigation used rabbit polyclonal antibodies against rat AR (Santa Cruz Biotechnology; CA, USA) and rabbit polyclonal antibodies against rat Wilms' tumor nuclear protein (Wt-1). A non-immune rabbit IgG was used as a negative control instead of primary polyclonal antibodies. After washing with phosphate-buffered saline (PBS), the sections were incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG for 30 minutes. Diaminobenzidine (DAB) was used as the substrate for visualization. The slides were then dehydrated, using ethanol and xylene, counterstained with hematoxylin, and mounted in resin.

Statistical Analyses

Analyses of variance (ANOVA) and *post-hoc* multiple comparisons Tukey's test were conducted based on Steel and Torrie methodology [22]. We used the statistical analysis system (SAS) program (Cary, NC, USA).

Results

Liver and Kidney Functions

Tables 2 and 3 indicate that alpha-chlorohydrin and exogenous testosterone significantly elevated serum ASAT, ALAT, GGT, and ALP activities, as well as urea, creatinine, and uric acid levels, compared with those of the control group.

Oxidative Stress Markers of Testis and Epididymis

Tables 4 and 5 indicate that ACH and exogenous testosterone significantly elevated MDA and NO levels while the CAT, SOD, and GSH levels were reduced significantly compared to those of the control group.

Serum Hormones and Enzymes

Tables 6 and 7 indicate that ACH and exogenous testosterone significantly reduced the serum ATPase, esterase, and G3PDH activities. Similar events occurred for testosterone, LH, and FSH levels compared to those of the control group.

Sperm Count and Motility

As shown in Table 8, ACH and exogenous testosterone caused a significant reduction in both sperm count, and the percentage of motile sperms. Additionally, there was a significant rise in the percentage of non-motile sperms compared to those of the control group. In the exogenous testosterone-treated group, all sperms became immotile.

Table 2. Effect of alpha–chlorohydrin and exogenous testosterone serum on the ALAT, ASAT, GGT, and ALP activity in Nile rats.

	ALAT (U/L)	ASAT (U/L)	GGT (U/L)	ALP (U/L)
Control	85.04 ± 6.32^{A}	89.42 ± 6.59^{A}	39.29 ± 2.25^{A}	148.38 ± 5.4^{A}
ACH-treated	95.57 ± 7.88^{BC}	110.7 ± 9.0^{C}	56.2 ± 3.4^{C}	160.45 ± 10.64^{C}
Testosterone-treated	108.9 ± 28^{D}	125.6 ± 19.9^{D}	65.28 ± 6.6^{D}	184.4 ± 13.1^{D}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P < 0.05$ based on the one-way ANOVA. Data appears as means and standard errors; ACH: Alpha–chlorohydrin. ALAT: Alanine Transaminase, GGT: gamma-glutamyl transferase, ALP: alkaline phosphatase, and ASAT: Aspartate Transaminase.

Table 3. Effect of alpha–chlorohydrin and exogenous testosterone on serum urea, creatinine, and uric acid in Nile rats.

	Urea (mg/dL)	Creatinine (mg/dL)	Uric acid (mg/dL)
Control	54.26 ± 3.13^{B}	1.4 ± 0.7^{B}	3.7 ± 1.15^{B}
ACH-treated	61.2 ± 3.2^{CD}	1.6 ± 0.6^{D}	4.68 ± 0.33^{D}
Testosterone-treated	60.12 ± 2.2^{C}	1.66 ± 0.06^{D}	4.86 ± 0.23^{D}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P<0.05$ based on the one-way ANOVA. Data appears as means and standard errors; ACH: Alpha-chlorohydrin.

Table 4. Effect of Alpha–chlorohydrin and exogenous testosterone on MDA, NO, GSH, SOD, and CAT testes in Nile rats.

Treatments	Testis MDA	Testis NO	Testis GSH	Testis CAT	Testis SOD
Control	7.15 ± 1.6^{A}	506.4 ± 90.3^{A}	1228 ± 98A	17.7 ± 1.6^{A}	10716 ± 1068^{A}
ACH-treated	45.23 ± 9.07^{DE}	2019 ± 116^{E}	188 ± 25D	12.62 ± 1.5^{CD}	$7400 \pm 877B^{C}$
Testosterone- treated	23.15 ± 2.2^{C}	2371 ± 120^{C}	137.6 ± 11 C	15.33 ± 1.09^{C}	6345.12 ± 610^{C}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P < 0.05$ based on the one-way ANOVA. Data appears as means and standard errors; ACH: Alpha–chlorohydrin. NO, nitric oxide; MDA, malondialdehyde; GSH, reduced glutathione; CAT, catalase SOD, superoxide dismutase.

Table 5. Effect of alpha–chlorohydrin and exogenous testosterone on epididymis MDA, NO, GSH, SOD and CAT in Nile rats.

Treatments	Epididymis MDA	Epididymis NO	Epididymis GSH	Epididymis CAT	Epididymis SOD
Control	6.61 ± 1.03^{A}	1724 ± 408^{A}	2011 ± 638^{A}	53.2 ± 6.02^{A}	15456 ± 521^{A}
ACH-treated	46.88 ± 14^{C}	3955 ± 144^{CD}	259 ± 46^{D}	16.46 ± 2.5^{D}	11966 ± 313^{D}
Testosterone-treated	35.39 ± 3.2^{E}	3003 ± 275^{E}	270.07 ± 24^{E}	15.83 ± 1.04^{E}	7146 ± 427^{E}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P < 0.05$ based on the one-way ANOVA. Data appears as means and standard errors; ACH: Alpha-chlorohydrin. NO, nitric oxide; MDA, malondialdehyde, SOD, superoxide dismutase; GSH, reduced glutathione; CAT, catalase.

Table 6. Effect of alpha–chlorohydrin and exogenous testosterone on serum testosterone, LH & FSH in Nile rats.

Treatments	Testosterone (mIU/ml)	LH (mIU/ml)	FSH (mIU/ml)
Control	0.80 ± 0.11^{A}	0.472 ± 0.34^{A}	0.462 ± 0.11^{A}
ACH-treated	0.154 ± 0.009^{C}	0.04 ± 0.01^{D}	0.11 ± 0.02^{C}
Testosterone-treated	1.68 ± 0.23^{D}	0.018 ± 0.003^{D}	0.054 ± 0.009^{D}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P < 0.05$ based on the one-way ANOVA. Data appear as means and standard errors; ACH: Alpha–chlorohydrin. FSH: follicular stimulating hormone; LH: Luteinizing hormone

Table 7. Effect of alpha–chlorohydrin and exogenous testosterone on serum ATPase, Esterase & G3PDH in Nile rats.

Treatments	ATPase (mU/mL)	Esterase (mU/mL)	G3PDH (mU/mL)
Control	3.2 ± 0.56^{A}	15.4 ± 4.2^{A}	21.7 ± 2.1^{A}
ACH-treated	0.91 ± 0.08^{BC}	5.46 ± 0.77^{C}	10.89 ± 0.51^{C}
Testosterone-treated	1.06 ± 0.13^{D}	8.13 ± 1.01^{D}	12.82 ± 2.2^{C}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P < 0.05$ based on the one-way ANOVA. Data appears as means and standard errors; ACH: Alpha–chlorohydrin. ATPase: adenosine triphosphate enzyme, G3PDH: glyceraldehyde 3-phosphate dehydrogenase

Table 8. Effect of alpha–chlorohydrin and exogenous testosterone on sperm count and motility in Nile rats

Treatments	Sperm count $x10^{6}$ /ml	Motile sperm (%)	Non-motile sperm (%)
Control	154 ± 12.1^{A}	62.8 ± 6.2^{A}	37.16 ± 6.2^{A}
ACH-treated	62.3 ± 11.8^{D}	6.33 ± 2.5^{CD}	93.6 ± 2.5^{D}
Testosterone-treated	22.6 ± 2.01^{D}	0.0 ± 0.0^{D}	100 ± 0.0^{D}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P < 0.05$ based on the one-way ANOVA. Data appears as means and standard errors; ACH: Alpha–chlorohydrin.

Table 9. Effect of alpha–chlorohydrin and exogenous testosterone on index weight of male reproductive organs in Nile rats.

Treatments	Testis	Epididymis	Prostate	Seminal vesical
Control	1.075 ± 0.06^{A}	0.285 ± 0.02^{A}	0.049 ± 0.002^{A}	0.529 ± 0.043^{A}
ACH-treated	0.904 ± 0.01^{B}	0.136 ± 0.05^{B}	0.077 ± 0.02^{BC}	0.25 ± 0.02^{BC}
Testosterone- treated	0.186 ± 0.017^{D}	0.136 ± 0.082^{B}	0.021 ± 0.004^{D}	0.19 ± 0.037^{C}

The Duncan post hoc test is used to determine if the means of the columns were significantly different at $P < 0.05$ based on the one-way ANOVA. Data appears as means and standard errors; ACH: Alpha–chlorohydrin.

General Health and Index Organ Weights

The rats that received both ACH and testosterone did not experience any mortality. The rats were active and displayed normal behavior. In both treatment groups, no unusual clinical symptoms were observed, except for a significant decrease in the index weight of reproductive organs compared to those of normal controls (Table 9).

Testicular Histological, Morphometric Analyses, and Johnsons' Score

Macroscopic examinations of the testes in the control group showed a normal testicular architecture with complete spermatogenesis. The testicular tissue consisted of round or oval-shaped seminiferous tubules separated by interstitial tissue (Figure 1a & 1b). In contrast, ACH-treated testes had been affected for their spermatogenesis. The testes displayed a thickened tunica albuginea, reduced diameter of seminiferous tubules, congested blood vessels, and spermatogenic arrest. There were degenerated seminiferous tubules with germ cells exfoliating or detached from the seminiferous epithelia. The seminiferous tubules lacked sperm, and wide intertubular spaces between seminiferous tubules were observed with large congested blood vessels (Figure 1c, 1d & 1e).

Histopathological changes in the testosterone-treated testes were significantly affected for their spermatogenesis. The testis exhibited thickening in the tunica albuginea accompanied by spermatogenic arrest, characterized by degeneration of all seminiferous tubules, absence of spermatogenic series in the tubular lumens, numerous congested blood vessels and interstitial edema (Figure 1f, 1g & 1h). The seminiferous tubules revealed exfoliated germinal epithelia in the lumens (Figure 1g). Several

atrophied seminiferous tubules with irregular outlines and decreased diameters were observed (Figure 1g). In both treated groups, there was a significant increase in tunica albuginea thickness and a reduction in germinal lining height and diameter of the seminiferous tubules compared to those of the untreated group (Figure 2a, 2b & 2c). The mean scores for testicular biopsies in both treated rats were significantly lower than those of the control group, indicating impaired spermatogenesis had occurred in both treated groups (Figure 2d).

Testicular Immunohistochemistry

Androgen Receptors

The testes in the control rats showed normal, strong expression of androgen receptors (AR) in Sertoli, Leydig, and peritubular cells, as shown in Figure 3a. In the ACH group, AR immunoreactivity was weak (Figure 3b). In contrast, Sertoli, Leydig, and peritubular cells, in the exogenous testosterone group showed negative expression of AR (Figure 3c).

Wilms' Tumor Nuclear Protein

The testis in the untreated rats showed a strong, extensive reaction and stained nuclei of Sertoli and peritubular cells for Wt-1, as shown in Figure 4a. In the ACH group, Wt-1 immunoreactivity showed weak reactions to Sertoli and peritubular cells (Figure 4b). Wilms' tumor protein expression was limited in the peritubular and Sertoli cells in the exogenous testosterone group (Figure 4c).

Epididymal Histological and Morphometric Analyses

The epididymis in the control rats had normal structures of the epididymal ducts containing numerous spermatozoa with normal sperm density in the lumen (Figure 5a & 5b). In contrast, the epididymal ducts in the ACH-treated group were devoid of sperm and contained cell debris. Vacuolization occurred in the epididymal lining (Figures 5c & 5d). In the testosterone-treated group, the epididymal ducts showed a scarce number of spermatozoa (hypospermia) and marked inflammation. There was expansion of interstitial tissue with mononuclear inflammatory cell infiltration and congested blood vessels (Figure 5e & 5f). In both treatment groups, morphometric analyses of the epididymis revealed insignificant changes in the epithelial height. However, there was a significant reduction in the diameter of epididymal ducts compared to those noted in the untreated group (Figure 6d & 6e).

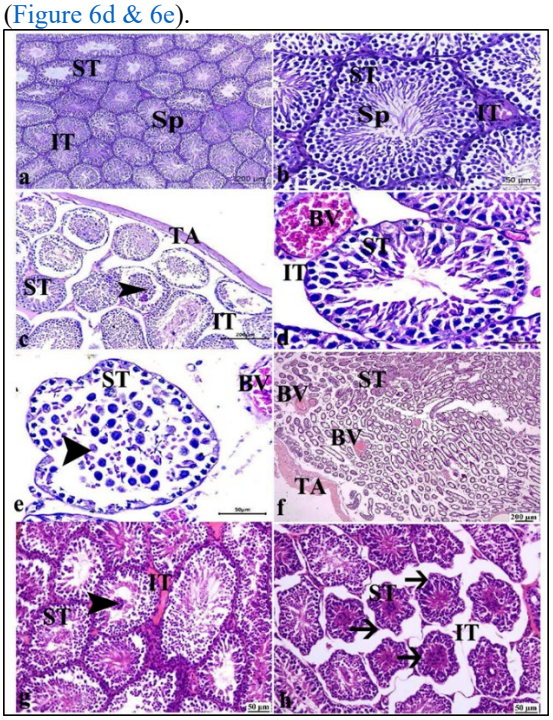

Figure 1. Photomicrographs of testis tissue sections from Nile grass rats stained with H&E.
(a & b) show the control group with a normal testicular structure characterized by oval or rounded seminiferous tubules (ST) containing numerous sperm (Sp) and narrow intertubular spaces (IT). (c, d & e) show the ACH-treated group which exhibits thickening of the tunica albuginea (TA), degenerated seminiferous tubules with exfoliation of germ cells (head arrow), wide intertubular spaces (IT), and large congested blood vessels (BV) (f, g & h), demonstrate the testosterone-treated group, displaying thickening of the tunica albuginea (TA), atrophied seminiferous tubules with irregular outlines (arrows), tubular degeneration of seminiferous tubules (ST), exfoliation of the germinal epithelium (head arrow), and interstitial edema with large intertubular spaces (IT).

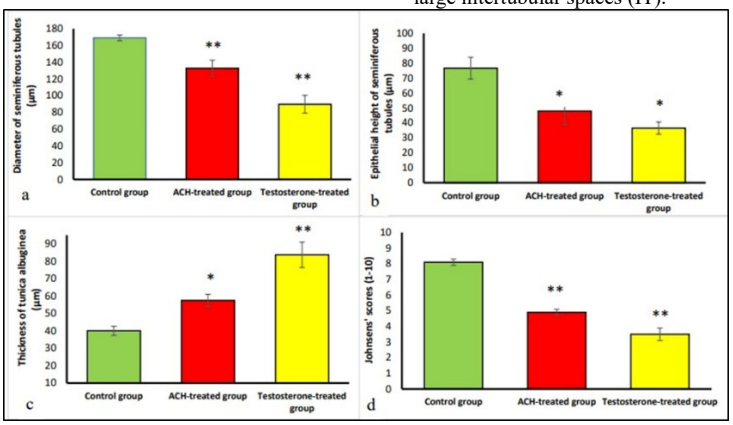

Figure 2. Effect of alpha-chlorohydrin and exogenous testosterone on testicular morphometric analysis and stages of spermatogenesis in Nile rats. (a, b & c) Morphometric analysis of the testis. a) Diameter of seminiferous tubules, b) Epithelial height of seminiferous tubules, c) Thickness of the tunica albuginea, and d) Stages of spermatogenesis. Data expressed as means ±SE were significantly different from those of the control group (*P<0.05 and **P<0.001).

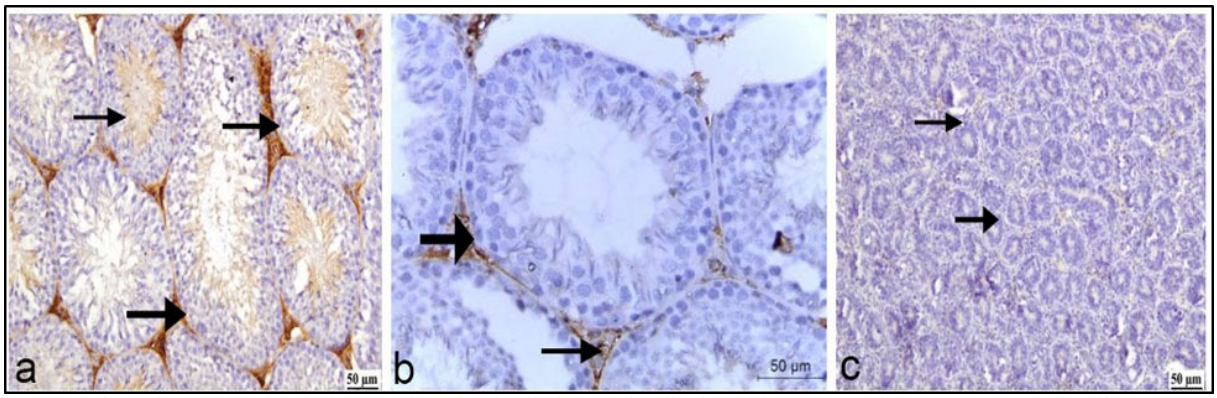

Figure 3. Photomicrographs of testis tissue sections showing the immunolocalization of the nuclear androgen receptor (AR) in Nile grass rats. (a) The control group displays brown immunohistochemical stains localized at nuclei of Leydig, Sertoli, and peritubular cells (arrows). (b) The ACH-treated group exhibits a weak immunoreaction of AR. The presence of degenerated seminiferous tubules reduces the number of necrotic Leydig cells (arrows). (c) The testosterone-treated group shows a negative AR immunoreaction. The number of necrotic Leydig cells is reduced by degenerated seminiferous tubules (arrows).

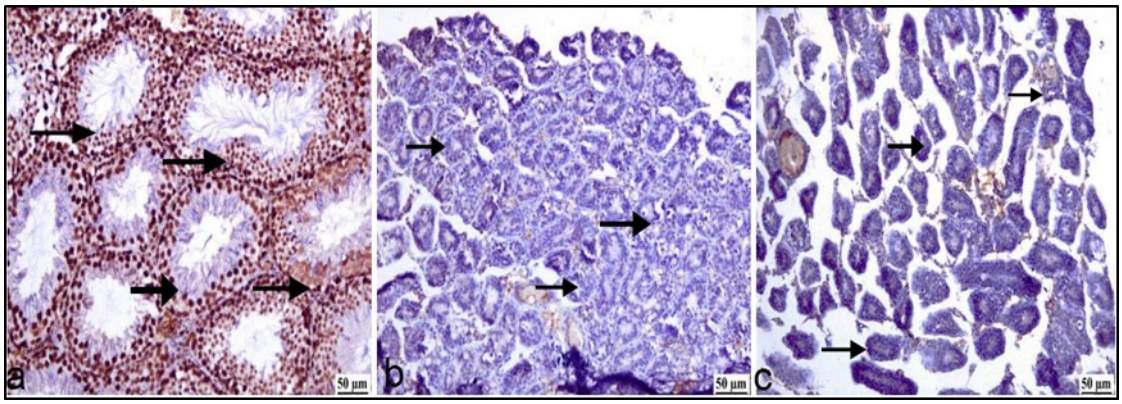

Figure 4. Photomicrographs of testis tissue sections showing the immunolocalization of Wilms' tumor protein 1(Wt-1) in Nile grass rats. (a) The control group shows a strong and extensive reaction of Wt-1 nuclei in the Sertoli and peritubular cells (arrows). (b) The ACH-treated group exhibits weak immunoreactions in the Sertoli cells and peritubular cells within degenerated seminiferous tubules (arrows). (c) The testosterone-treated group demonstrates a limited and negative immunoreaction in the Sertoli cells and peritubular cells within degenerated seminiferous tubules (arrows).

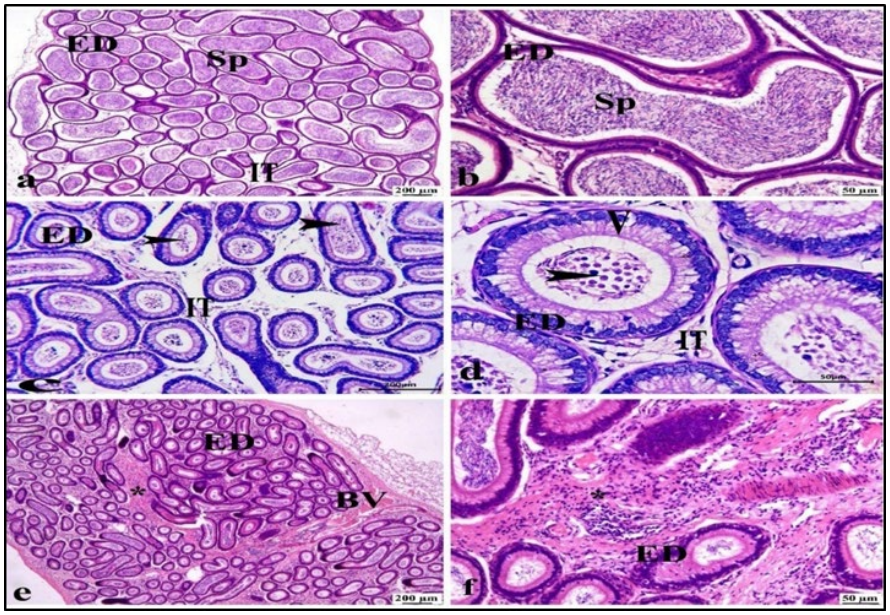

Figure 5. Photomicrographs of epididymal tissue sections from Nile rats stained with H&E. (a & b) The control group demonstrates the normal histological structure of the epididymis, with epididymal ducts (ED) containing a normal density of spermatozoa in the ductal lumens (Sp), and narrow interstitial spaces (IT). (c & d) The ACH-treated group shows shrinkage in the epididymal ducts, the presence of cellular debris (head arrows), vacuolated epithelial cells (v), and epididymal ducts devoid of spermatozoa. (e & f) The testosterone-treated group shows epididymal ducts (ED) with a low number of spermatozoa (hypospermia), significant inflammation characterized by expansion of interstitial tissue and infiltration of mononuclear inflammatory cells (*), and congested blood vessels (BV).

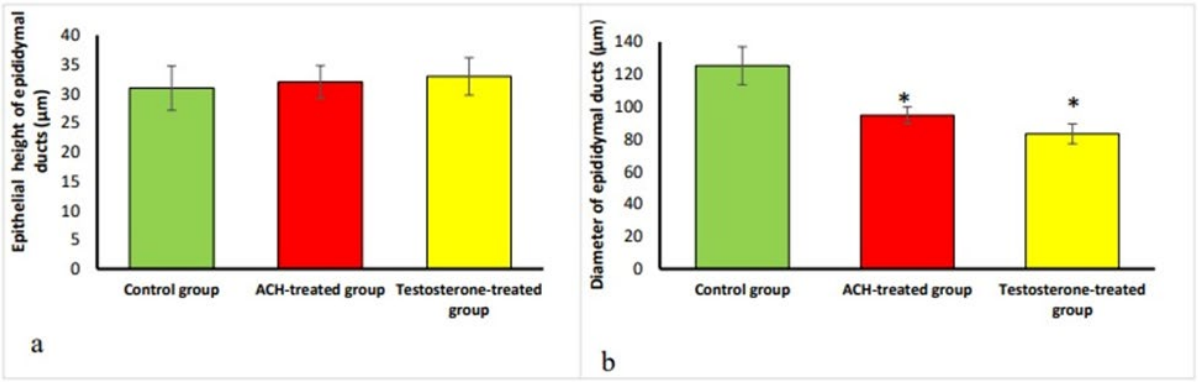

Figure 6. Effect of alpha-chlorohydrin and exogenous testosterone on morphometric analysis of the epididymis in Nile rats. a) Epithelial height of epididymal ducts, and b) Diameter of epididymal ducts. Data expressed as means ± SE were significantly different from those of the control group (*P<0.05).

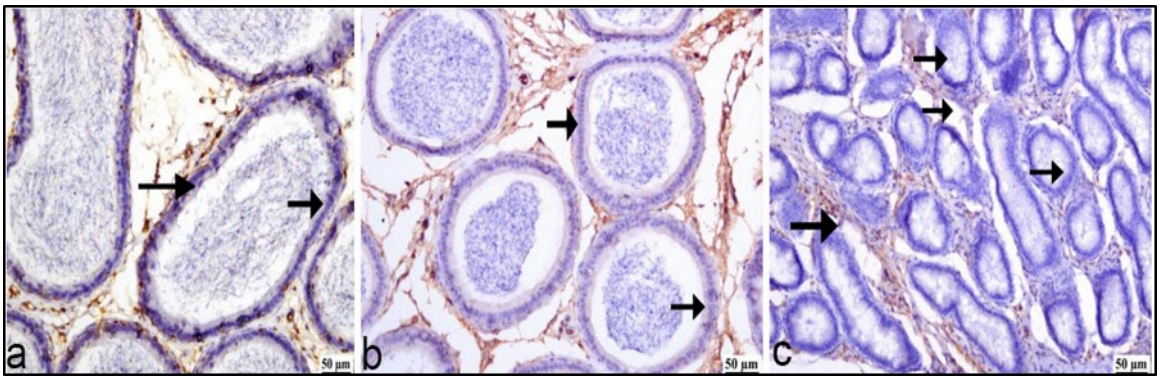

Figure 7. Photomicrographs of epididymis tissue sections showing the immunolocalization of the nuclear androgen receptor (AR) in Nile grass rats. (a) In the control group, a strong nuclear AR immunoreaction was observed in the epididymal ducts and intertubular spaces (arrows). (b) The ACH-treated group exhibited a weak AR immunoreaction in the epididymal ducts and intertubular spaces (arrows). (c) The testosterone-treated group displayed a weak AR immunoreaction in the shrinkage-cribriform shape of the epididymal ducts and intertubular spaces (arrows).

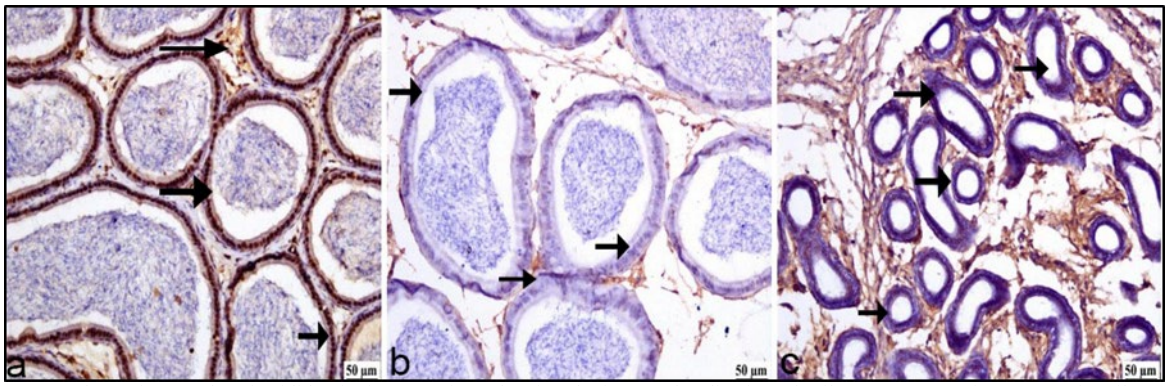

Figure 8. Photomicrographs of epididymis tissue sections showing the immunolocalization of Wilms' tumor protein 1 (Wt-1) in Nile grass rats. In the control group (a) there is a strong nuclear immunoreaction of Wt-1) in the epididymal ducts and intertubular spaces (arrows). In the ACH-treated group (b) the nuclear immunoreaction of Wt-1 is weak in both the epididymal ducts and intertubular spaces (arrows). In the testosterone-treated group (c) there is a limited nuclear immunoreaction of Wt-1 in the epididymal ducts and intertubular spaces (arrows).

Epididymal Immunohistochemistry

Androgen Receptors

In the control group, strong nuclear AR immunoreactivity was observed in epididymal ducts and intertubular spaces (Figure 7a). After treatment; however, the ACH-treated group showed weak AR immunoreactivity in these areas (Figure 7b), while, the testosterone-treated group showed negative AR immunoreactivity (Figure 7c).

Wilms' Tumor Nuclear Protein

In terms of Wt-1 expression, the control group exhibited strong nuclear immunoreaction in the epididymal ducts and intertubular spaces (Figure 8a). Conversely, the ACH-treated group showed weak immunoreaction in these areas (Figure 8b). The testosterone-treated group displayed limited expression of Wt-1 immunoreaction, mainly in the cribriform shape of the epididymal ducts and intertubular spaces (Figure 8c).

Discussion

This study investigated the potential of the anti-fertility effects of ACH and exogenous testosterone in adult Nile grass rats, to integrate these agents into rodent pest management programs. The results showed a strong impact on male fertility, but no recorded deaths, indicating no overall health impact. There was evidence of hepato-renal damage induced by ACH and exogenous testosterone as shown by increased activities of serum ASAT, ALAT, GGT, and ALP, plus uric acid, creatinine, and urea. Furthermore, both treatments were found to raise the oxidative stress biomarkers in the testes and epididymis, which is consistent with the findings of previous studies [23, 24]. The antioxidant defense system is activated when there is severe impairment of oxidative stress and reactive oxygen species (ROS) production. The ROS damages spermatozoa through lipid peroxidation and disrupting the sperm function. Animals treated with ACH exhibited a dose-dependent decline in antioxidant enzymes, such as GSH, SOD, and catalase. Also, ACH increased the MDA and NO levels in the testes and epididymis. By reducing the activity of antioxidant enzymes, ACH promotes lipid peroxidation in the testis and epididymis leading to excessive ROS generation.

Both anti-fertility compounds led to significantly low levels of testosterone, LH, FSH, ATPase, Esterase, and G3PDH. Sperm pathology is associated with ROS production and oxidative damage, including depletion of the above parameters. These findings are consistent with those of Kim, *et al*. [23]. Of note, ACH inhibits GAPDH by oxidizing it within the sperm cells to form

3- chloroacetaldehyde [24, 25]. GAPDH, a glycolytic enzyme essential for male fertility and sperm motility is a vital target protein in oxidative stress [26]. Additionally, sperms contain an enzyme isoform that is highly susceptible to oxidative damage [27]. ACH inhibits GAPDH activity in rat sperm and epididymis by depleting ATP levels [3, 27]. The spermatogenic effects of ACH have been attributed to the blockage of epididymal sperm metabolism [3], inhibition of androgen-dependent enzymes, like acetylcholine esterase and adenosine triphosphatase within the epididymis [28], and inhibition of sperm motility through prolonged alkylation of cysteine residues [29]. A low sperm count is associated with low testosterone levels, and a free-radical attack can reduce their motility [30]. Further, impaired sperm motility may lead to infertility as sperms are unable to penetrate the zona pellucida and reach the site of fertilization [31].

In the current study, both anti-fertility compounds caused a significant decline in the male reproductive organs of the treated rats compared to that of the control group. This decline is likely due to androgen insufficiency, as androgens are necessary for the normal growth of these organs [4, 5]. The testicular examinations revealed a significant decrease in John's score of spermatogenesis and changes in histology, including tubular atrophy and Sertoli cell necrosis. These were evident by a significant decrease in the diameter of seminiferous tubules after exposure to ACH and exogenous testosterone. These results are consistent with other recent studies in experimental animals exposed to ACH [3, 4].

Compared to the control group, ACH and exogenous testosterone decreased AR immune expression. Both groups also reduced the testosterone content in the testes, which led to a decrease in AR immunoexpression. Wahab, *et al*. [32] stated that lower serum testosterone levels results in a reduction in testicular AR expression in rats exposed to other chemicals. Also, there was a notable decline in the serum testosterone levels, corresponding to a reduction in mRNA expression of AR and genes responsible for regulating testosterone synthesis. Consequently, this led to malfunctions in the spermatogenesis [32]. The Wt-1-positive cells were mainly in Sertoli cells and were slightly reduced in the treated group compared to that of the control group. These results are consistent with previous studies that found reduced AR and Wt-1 positive staining in the treated compared to the untreated groups [32, 33].

The epididymis is an androgen-dependent organ. In both treatment groups, we found damages had occurred to the tissue cellular structures. The morphometric analyses of this organ showed the formation of intraepithelial spaces and vacuoles in the epithelial lining of the ducts, the presence of cellular debris inside the epididymal lumen, and vesicular congestion. These results may be attributed to the toxins that directly disrupt the structural and functional elements [34].

Exogenous testosterone causes hyperplasia of epididymal lining in rats, similar to other steroid hormones such as estrogen, which binds to its nuclear receptors [35]. As a result, gene expression regulated by transcription factors, including apoptosis, metabolism, cell proliferation, and differentiation, was activated [36]. Androgens play a crucial role in the maturation of spermatozoa in the epididymis by mediating the effects of androgen receptor (AR). Epididymal epithelial and interstitial cells contain the AR [36]. In this study, the epididymis in the control rats showed the same localization of androgen receptors in the control animals as observed in other studies [33]. The immunostaining AR and Wt-1 in the epididymal ducts in both treated rats revealed weak to negative reactions in the experimental groups. These results are consistent with those reported by other studies on a variety of biological markers [34].

Based on the present physiological, histological, and immune-histochemical findings, both ACH and exogenous testosterone can exert negative effects on

the male fertility of Nile grass rats. Thus, we recommend the application of these compounds as part of an integrated pest management program.

Conflict of Interests

The authors declared no conflicts of interest.

Funding

This study did not receive funding from any external agency.

Acknowledgements

The authors wish to acknowledge the support of the faculty, management and staff of the following academic institutions: 1) Agriculture Research Center, Doki, Egypt;

2) Zoology Department, Faculty of Science, Al-Azhar University, Assuit, Egypt; and 3) Zoology Department, Faculty of Science, Ain-shams University, Cairo, Egypt.

Ethical Considerations

This study was conducted based on the guidelines of the Ethics Committee, Faculty of Science, Al-Azhar University, Assuit (approval #: AZHAR,11/2022).

Authors' Contributions

Conceptualization: Ayat Taha, Mahmoud Ashry, Magdy Wilson, Mohsen A. Moustafa, and Zeinab Hassan. Methodology, Investigation, and Writing-original draft: Ayat Taha; Histopathology & Immunohistochemistry: All authors; Supervision, Writing, Review and Editing: Ayat Taha, Mahmoud Ashry, Magdy Wilson, Mohsen A. Moustafa, and Zeinab Hassan.

References

1. Taha A. Assessment of non-target toxicity of profenofos insecticide on the aquatic bird; the white egret, Egretta alba. Egyptian Journal of Aquatic Biology and Fisheries. 2022;**26**(2):263-76. [**doi:** 10.21608/ejabf.2022.228725]
2. Taha A, Soliman S. Effect of α-Chlorohydrin water-bait on the fertility of captive males of the Egyptian fruit-bat (Rousettus aegyptiacus) and the proper time for controlling its free-ranging populations in Egypt. Egyptian Journal of Aquatic Biology and Fisheries. 2019;**23**(4):227-37. [**doi:** 10.21608/ejabf.2019.53599]
3. Mahmoud YI, Taha A, Soliman S. 3-Monochloropropane-1,2-diol (alpha-chlorohydrin) disrupts spermatogenesis and causes spermatotoxicity in males of the Egyptian fruit-bat (Rousettus aegyptiacus). Biotech Histochem. 2018;**93**(4):293-300. [**doi:** 10.1080/10520295.2018.1437471] [**pmid:** 29595063]
4. Taha A, A. Gouda S. Antifertility Potential of n-Butanol and Ethyl Acetate Extracts of Penicillium oxalicum OM282858 in Male Albino Rats as Biological Control Agents. Journal of Experimental Biology and Agricultural Sciences. 2022;**10**(6):1354-65. [**doi:** 10.18006/2022.10(6).1354.1365]
5. Slott VL, Suarez JD, Simmons JE, Perreault SD. Acute inhalation exposure to epichlorohydrin transiently decreases rat sperm velocity. Fundam Appl Toxicol. 1990;**15**(3):597-606. [**doi:** 10.1016/0272-0590(90)90044-k] [**pmid:** 2258022]
6. Lubicz-Nawrocki CM, Chang MC. The onset and duration of infertility in hamsters treated with alpha-chlorohydrin. J Reprod Fertil. 1974;**39**(2):291-5. [**doi:** 10.1530/jrf.0.0390291] [**pmid:** 4416391]
7. Soliman S, Mahmoud Y, Taha A. Evaluating the Efficacy of the Male Chemosterilant Alpha-Chlorohydrin on Three Egyptian Wild Rodent Pests under Laboratory Conditions. Egyptian Journal of Zoology. 2016;**66**(66):71-84. [**doi:** 10.12816/0034709]
8. Basaria S. Reproductive aging in men. Endocrinol Metab Clin North Am. 2013;**42**(2):255-70. [**doi:** 10.1016/j.ecl.2013.02.012] [**pmid:** 23702400]
9. Kalfa N, Gaspari L, Ollivier M, Philibert P, Bergougnoux A, Paris F, et al. Molecular genetics of hypospadias and cryptorchidism recent developments. Clin Genet. 2019;**95**(1):122-31. [**doi:** 10.1111/cge.13432] [**pmid:** 30084162]
10. Yokonishi T, McKey J, Ide S, Capel B. Sertoli cell ablation and replacement of the spermatogonial niche in mouse. Nat Commun. 2020;**11**(1):40. [**doi:** 10.1038/s41467-019-13879-8] [**pmid:** 31896751]
11. Wen Q, Wang Y, Tang J, Cheng CY, Liu YX. Sertoli Cell Wt1 Regulates Peritubular Myoid Cell and Fetal Leydig Cell Differentiation during Fetal Testis Development. PLoS One. 2016;**11**(12):e0167920. [**doi:** 10.1371/journal.pone.0167920] [**pmid:** 28036337]
12. Hentrich A, Wolter M, Szardening-Kirchner C, Luers GH, Bergmann M, Kliesch S, et al. Reduced numbers of Sertoli, germ, and spermatogonial stem cells in impaired spermatogenesis. Mod Pathol. 2017;**30**(2):311. [**doi:** 10.1038/modpathol.2016.194] [**pmid:** 28144033]
13. Taha A. Laboratory and histological studies on the use of the male anti-fertility agent alpha-chlorohydrin in the control of some wild vertebrate pests in Egypt: Faculty of Science Ain Shams University; 2016.
14. Callies F, Kollenkirchen U, von zur Muhlen C, Tomaszewski M, Beer S, Allolio B. Testosterone undecanoate: a useful tool for testosterone administration in rats. Exp Clin Endocrinol Diabetes. 2003;**111**(4):203-8. [**doi:** 10.1055/s-2003-40464] [**pmid:** 12845558]
15. Schumann G, Klauke R. New IFCC reference procedures for the determination of catalytic activity concentrations of five enzymes in serum: preliminary upper reference limits obtained in hospitalized subjects. Clin Chim Acta. 2003;**327**(1-2):69-79. [**doi:** 10.1016/s0009-8981(02)00341-8] [**pmid:** 12482620]
16. Methods for measurement of catalytic concentration of enzymes, Part 4.IFCC method for γ- glutamyltransferase. Journal of Clinical Chemistry and Clinical Biochemistry. 1983;**21**(10):633-46. [**doi:** 10.1515/cclm.1983.21.10.633]
17. Moss AR, Henderson DW. Henderson Clinical enzymology. In: Burtis CA, Ashwood ER, Tietz NW, editors. Tietz textbook of clinical chemistry. 3th ed. Philadelphia: W.B Saunders Company; 1999.
18. Chaney AL, Marbach EP. Modified reagents for determination of urea and ammonia. Clin Chem. 1962;**8**:130-2. [**pmid:** 13878063]
19. Husdan H, Rupoport A. Estimation of creatinine by Jaffesreactions Comparison of threemethod. Clinical Chemistry. 1969;**138**:459-70.
20. Stong L, Berta E, Rebar L, Trivedi RC. New enzymatic method for serum uric acid at 500 nm. Clinical Chemistry. 1978;**24**(11):1908-11. [**doi:** 10.1093/clinchem/24.11.1908]
21. Johnsen SG. The stage of spermatogenesis involved in the testicular-hypophyseal feed-back mechanism in man. Acta Endocrinol (Copenh). 1970;**64**(2):193-210. [**doi:** 10.1530/acta.0.0640193] [**pmid:** 5468365]
22. Steel RGD, Torrie JH. Principles and procedures of statistics. London: McGraw-Hill Book Co.,; 1960.
23. Kim SH, Lee IC, Lim JH, Moon C, Bae CS, Kim SH, et al. Spermatotoxic effects of alpha-chlorohydrin in rats. Lab Anim Res. 2012;**28**(1):11-6. [**doi:** 10.5625/lar.2012.28.1.11] [**pmid:** 22474469]
24. Xing HZ, Fang B, Pang GF, Ren FZ. 3-Monochloropropane-1, 2-diol causes irreversible damage to reproductive ability independent of hormone changes in adult male rats. Food Chem Toxicol. 2019;**124**:10-6. [**doi:** 10.1016/j.fct.2018.11.023] [**pmid:** 30453003]
25. Cooney SJ, Jones AR. Inhibitory effects of (S)-3-chlorolactaldehyde on the metabolic activity of boar spermatozoa in vitro. J Reprod Fertil. 1988;**82**(1):309-17. [**doi:** 10.1530/jrf.0.0820309] [**pmid:** 3339589]

26. Miki K, Qu W, Goulding EH, Willis WD, Bunch DO, Strader LF, et al. Glyceraldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycolytic enzyme, is required for sperm motility and male fertility. Proc Natl Acad Sci U S A. 2004;**101**(47):16501-6. [**doi:** 10.1073/pnas.0407708101] [**pmid:** 15546993]
27. Jelks K, Berger T, Horner C, Miller MG. alpha-chlorohydrin induced changes in sperm fertilizing ability in the rat: association with diminished sperm ATP levels and motility. Reprod Toxicol. 2001;**15**(1):11-20. [**doi:** 10.1016/s0890-6238(00)00115-5] [**pmid:** 11137374]
28. Jelks KB, Miller MG. alpha-Chlorohydrin inhibits glyceraldehyde-3-phosphate dehydrogenase in multiple organs as well as in sperm. Toxicol Sci. 2001;**62**(1):115-23. [**doi:** 10.1093/toxsci/62.1.115] [**pmid:** 11399799]
29. Kalla NR, Singh B. Synergistic effect of alpha chlorohydrin on the influence of copper ions on human spermatozoa. Int J Fertil. 1981;**26**(1):65-7. [**pmid:** 6113213]
30. Kao SH, Chao HT, Chen HW, Hwang TIS, Liao TL, Wei YH. Increase of oxidative stress in human sperm with lower motility. Fertil Steril. 2008;**89**(5):1183-90. [**doi:** 10.1016/j.fertnstert.2007.05.029] [**pmid:** 17669405]
31. Aly HA, Domenech O, Abdel-Naim AB. Aroclor 1254 impairs spermatogenesis and induces oxidative stress in rat testicular mitochondria. Food Chem Toxicol. 2009;**47**(8):1733-8. [**doi:** 10.1016/j.fct.2009.03.019] [**pmid:** 19306909]
32. Wahab OA, Princely AC, Oluwadamilare AA, Ore-Oluwapo DO, Blessing AO, Alfred EF. Clomiphene citrate ameliorated lead acetate-induced reproductive toxicity in male Wistar rats. JBRA Assist Reprod. 2019;**23**(4):336-43. [**doi:** 10.5935/1518-0557.20190038] [**pmid:** 31173495]
33. Zhu LJ, Hardy MP, Inigo IV, Huhtaniemi I, Bardin CW, Moo-Young AJ. Effects of androgen on androgen receptor expression in rat testicular and epididymal cells: a quantitative immunohistochemical study. Biol Reprod. 2000;**63**(2):368-76. [**doi:** 10.1095/biolreprod63.2.368] [**pmid:** 10906039]
34. Elbakry R, Ibrahim M. Histological, immunohistochemical and biochemical study of the effect of triclosan and its withdrawal on cauda epididymis of adult albino rat. Egyptian Journal of Histology. 2019;**42**(1):84-98. [**doi:** 10.21608/ejh.2018.5866.1032]
35. Milgrom E, Thi L, Atger M, Baulieu EE. Mechanisms regulating the concentration and the conformation of progesterone receptor(s) in the uterus. J Biol Chem. 1973;**248**(18):6366-74. [**pmid:** 4354209]
36. Nelson PS, Clegg N, Arnold H, Ferguson C, Bonham M, White J, et al. The program of androgen-responsive genes in neoplastic prostate epithelium. Proc Natl Acad Sci U S A. 2002;**99**(18):11890-5. [**doi:** 10.1073/pnas.182376299] [**pmid:** 12185249]