


Impact of pubertal imidacloprid exposure on the genital tract of Japanese quail (*Coturnix Coturnix japonica*): Histomorphometric and ultrastructural study

Mohammed I.A. Ibrahim^{a,*} , Rephima M. Phaswane^a, Antoinette V. Lensink^b, Christo J. Botha^a

^a Department of Paraclinical Sciences, Faculty of Veterinary Science, University of Pretoria, South Africa

^b Electron Microscope Unit, Department of Anatomy and Physiology, Faculty of Veterinary Sciences, University of Pretoria, South Africa

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ABSTRACT

Background: The effects of exposure to the neonicotinoid pesticide, imidacloprid (IMI), on the histopathology and ultrastructure of the genital tracts of pubertal male and female Japanese quails (*Coturnix Coturnix japonica*) were investigated.

Methods: Pubertal quail ($n = 28$), commencing at 5 weeks post-hatching, were orally gavaged with 1.55 (low), 3.1 (medium), and 6.2 (high) mg/kg IMI, dissolved in distilled water, twice a week for four weeks. Control birds received distilled water only. Samples collected from male and female genital tracts included epididymal duct and magnum and shell gland. Histopathology, histometric measurements, as well as transmission and scanning electron microscopy were performed.

Results and conclusion: The medium and high IMI doses induced pathomorphological changes in the epithelial cells of the magnum and shell gland, which included a ciliary loss, presence of intracytoplasmic vacuoles, mitochondrial damage, and fewer secretory granules. In addition, there were cytoplasmic vacuoles detected in the efferent ducts and epididymal ducts of quails treated with medium and high IMI doses. The epithelial heights of the magnum, shell gland, rete testis and proximal efferent duct were reduced ($P \leq 0.05$) in all IMI treatment groups. In addition, the epididymal duct epithelial heights decreased ($P \leq 0.05$) at medium and high IMI doses, but changes in the distal efferent ducts were only observed in the high dose group. Overall, the results confirmed that pubertal exposure to IMI induced dose-dependent pathomorphological changes in male and female quail genital tracts, suggesting that IMI might impact their fertility and reproductive function.

1. Introduction

The Japanese quail (*Coturnix Coturnix japonica*) serves as a common experimental avian model that is utilized in various fields, such as developmental biology, endocrinology, ageing, immunology and behavioural studies (Baer et al., 2015). In addition, Japanese quails are also useful to assess the impact of environmental contaminants (Alam and Kurohmaru, 2021; Molele et al., 2023; Zakariah et al., 2022). Due to their relative short lifespan, ease of breeding in laboratory facilities, the modest size of breeding adults, quails are a well-studied model in comparative pathology and have been used in laboratory experimental settings (Baer et al., 2015). Furthermore, quails are economically important to the food industry, contributing 10% and 2% to global

table egg and meat production, respectively (Lukanov, 2019).

Imidacloprid (IMI) is a neonicotinoid insecticide, widely used in agriculture for pest control and in veterinary medicine for topical treatment of flea infestation in dogs and cats (Goulson, 2013). Despite being banned for outdoor use by the European Union (EU) and the United Kingdom (UK) since 2018 (European Commission, 2022), IMI is commonly detected in fruit, vegetables, seeds, and surface drinking water (Thompson et al., 2020). Consequently, IMI poses a higher risk of exposure to non-target organisms (Nugnes et al., 2023; Zhao et al., 2020), especially birds via IMI-treated seeds and contaminated water (Millot et al., 2017; Poliserpi et al., 2021). Pandey et al. (2017) reported that IMI disrupts the endocrine system of red munia (Asian strawberry finch), causing hormonal imbalances in the pituitary gland and testis.

* Corresponding author.

E-mail addresses: wadibrahim352@gmail.com, u17372098@tuks.co.za (M.I.A. Ibrahim).

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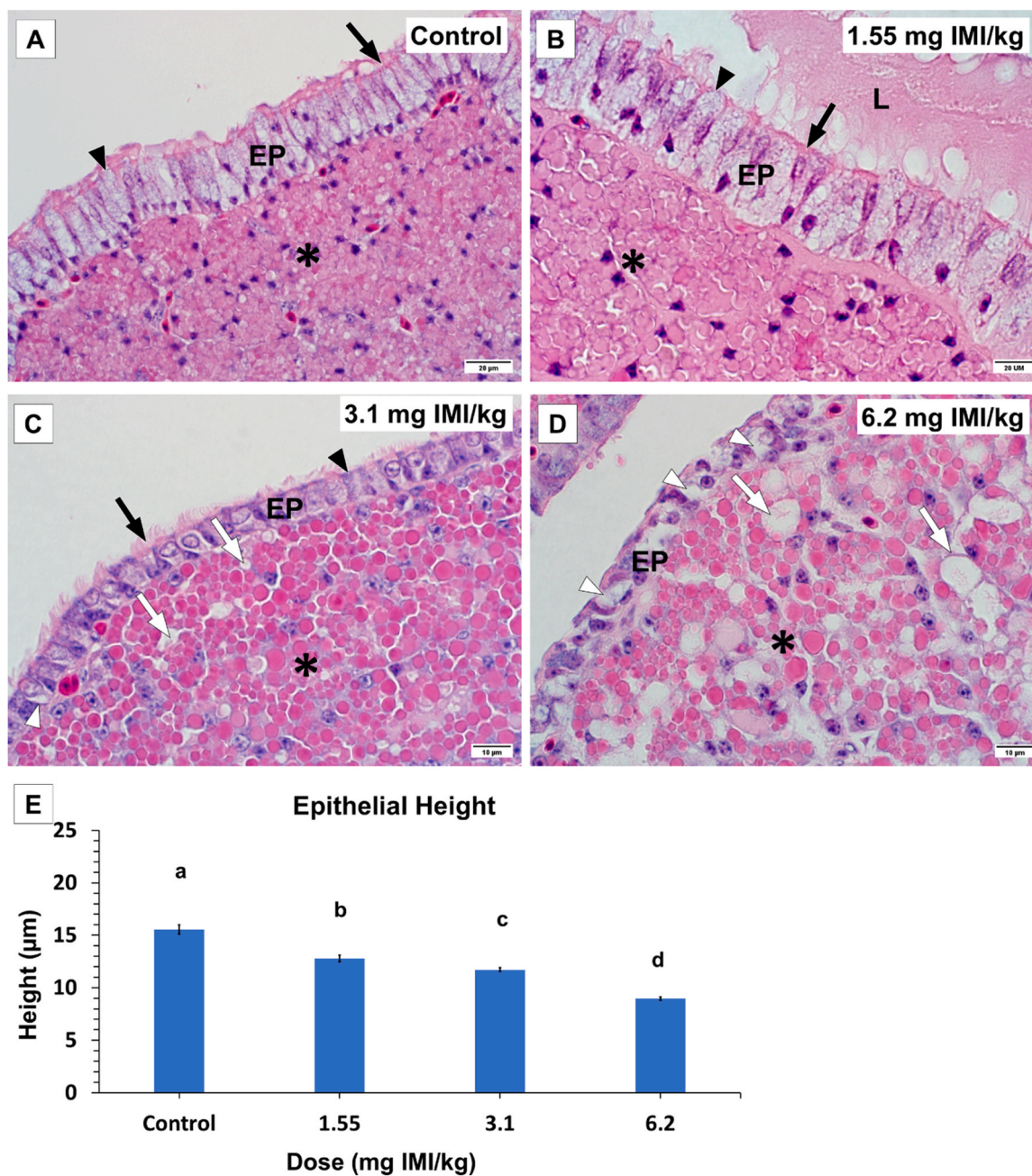


Fig. 1. Light micrographs of the magnum in Japanese quails following imidacloprid (IMI) exposure. (A) control, and (B) low (1.55 mg/kg), (C) medium (3.1 mg/kg) and (D) high (6.2 mg/kg) IMI-dosage groups. Simple columnar epithelium (Ep) contains ciliated (black arrows) and nonciliated (black arrowheads) cells. Asterisks: tubular gland. There are numerous vacuoles in the epithelium (white arrowheads) and gland (white arrows). L: Lumen. (E) Epithelial height of the magnum of Japanese quail ($n = 4$ per group) following treatment with low, medium, and high doses of IMI. Significant differences ($P \leq 0.05$) between groups are indicated by (a, b, c, d).

Recently, there has been increased interest in clarifying the toxicological mechanisms by which environmental pollutants induce reproductive toxicity (Gautam et al., 2024). Imidacloprid induces endocrine disruption and interferes with various hormones and physiological functions (Mikolić and Karačonji, 2018). Previous studies have concluded that IMI might have adverse impacts on reproductive functions (Al-Awar, 2021; Bal et al., 2012; Lovaković et al., 2021; Nabiuni et al., 2015; Yuan et al., 2020). Severe damage to testicular morphology, as well as a decrease in testosterone concentrations and androgen receptors, were reported in mice following exposure to IMI (Yuan et al., 2020). Soujanya et al. (2022) suggested that IMI could disrupt the estrous cycle and decrease estrogen concentrations in rats. In addition,

Saber et al. (2021) reported that exposure of rats to IMI at a dose of 22.5 mg/kg caused oxidative stress in the testicular tissue by increasing malondialdehyde levels and decreasing total glutathione levels. Increased activities of catalase (CAT), superoxide dismutase (SOD), and total peroxidases were also detected in rats after exposure to 22.5 mg/kg of IMI (Tetsatsi et al., 2019). Similarly, an increase in lipid peroxidation activity and a decrease in CAT, glutathione peroxidase, glutathione reductase and SOD activities have been reported in the ovaries of rats after treatment with 20 mg/kg of IMI for 90 days (Kapoor et al., 2011). However, information on IMI-induced toxicity on the avian genital tract remains poorly documented.

In birds, the reproductive tract is comprised of a developed left

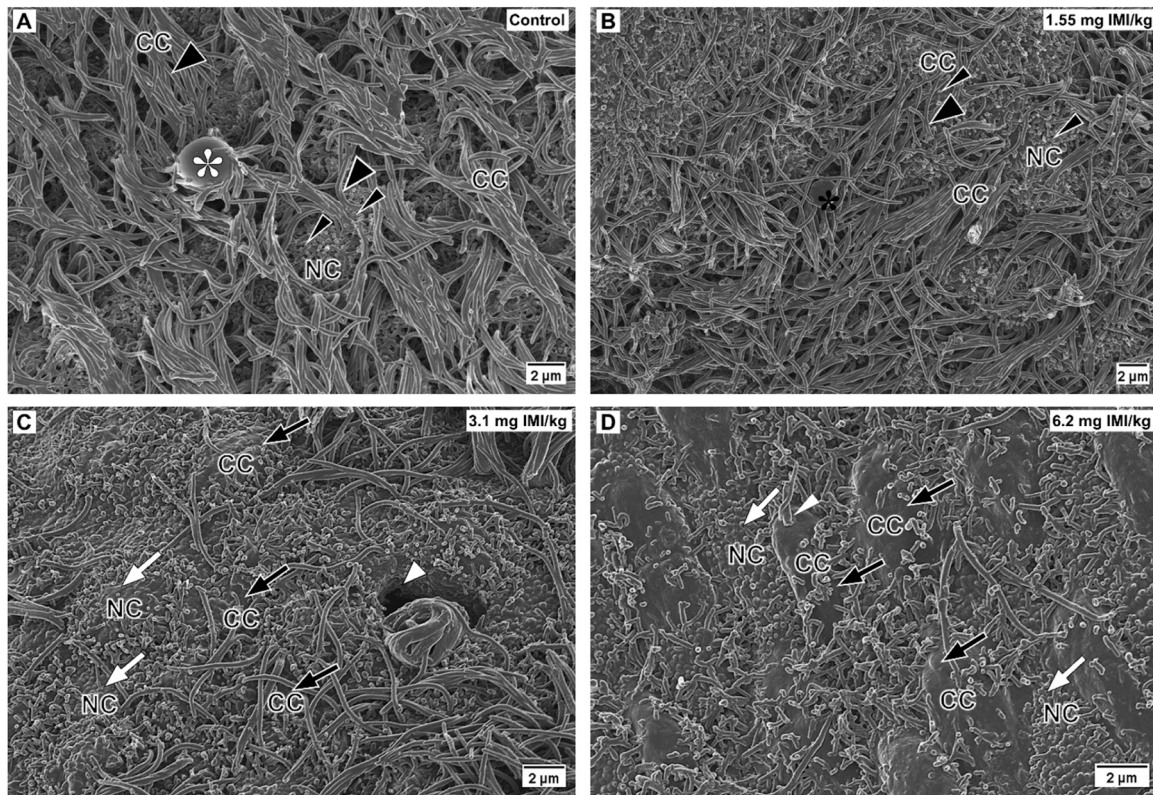


Fig. 2. Scanning electron micrographs of the magnum in Japanese quails following imidacloprid (IMI) treatment. (A) control, and (B) low (1.55 mg/kg), (C) medium (3.1 mg/kg) and (D) high (6.2 mg/kg) IMI-dosage groups. CC: ciliated cells. NC: nonciliated cells. Thick black arrowheads: cilia. Thin black arrowheads: microvilli. Asterisks: secretory granule. (C) Ciliated cells display a few cilia and short microvilli (black arrows) and nonciliated cells with few microvilli (white arrows). White thick arrowhead: glandular opening. (D) White arrows indicate nonciliated cells with short, swollen microvilli. Black arrows indicate ciliary loss in the ciliated cells with microvilli. White thin arrowhead: swollen cilium.

oviduct in females and an excurrent duct system in males (Hrabia, 2022). The oviduct contains the infundibulum, magnum, isthmus, uterus with shell gland, and vagina (Hrabia, 2022). After fertilization, in the infundibulum, the capturing and deposition of albumen, shell membranes, and eggshell occurs in sequence further down in the oviduct (Hrabia, 2022). The avian male reproductive tract, consists of the epididymal region and ductus deferens (Aire and Josling, 2000), which contributes to the improvement of spermatozoal motility, maturity, viability, as well as storage (Clulow and Jones, 1982). The epithelia of the oviduct and epididymal region contain estrogen receptors, which are responsible for regulating their functions (Ashoori et al., 2021; Madekurozwa, 2004; Niranjan and Srivastava, 2019; Oliveira et al., 2011). As IMI has antiestrogenic effects (Zhang et al., 2020), the reproductive tract may be susceptible.

Furthermore, the use of the IMI-coated seeds have been reported to pose a significant risk to farmed birds (Poliserpi et al., 2021), and consumption of even a small number of the treated seeds can lead to mortality or reproductive toxicity (Gibbons et al., 2015). In addition, Hallmann et al. (2014) reported a 3.5 % decline in wild bird populations in areas where IMI concentration exceeded 0.02 mg/L in surface water. Imidacloprid reduces egg production and delays egg lay onset in birds (Lopez-Antia et al., 2015), thus contributing to economic losses. Quails are also useful as bioindicators of environmental contamination, including a contaminant such as IMI (Osman et al., 2023). Therefore, the Japanese quail was chosen as an appropriate model for investigating morphological changes in the magnum, shell gland, and epididymal regions after exposure during puberty to various doses of IMI. The results from this study may provide insight into broader ecosystem health concerns associated with IMI.

2. Materials and methods

2.1. Animals and experimental design

Twenty-eight Japanese quails (*Coturnix Coturnix japonica*), aged three-week-old, were obtained from a commercial farm at Pretoria, South Africa (Zelda Enslin). The quails were maintained in a photoperiod of 16 h light: 8 h dark, at an ambient temperature of $25 \pm 2^\circ\text{C}$, with a relative humidity of $50\% \pm 5\%$ in a controlled environment at Poultry Research Facility, Faculty of Veterinary Science, University of Pretoria, until the age of 10 weeks. During the experiments, birds had free access to a commercial ration (Alzu Voere Feeds, Alzu Feeds) and water. The study was conducted following the approval of the South African National Department of Agriculture, Land Reform, and Rural Development (DALRRD), approval number: 2860ZY, as well as Research and Animal Ethics Committees of the University of Pretoria under the Protocol number: 003-23. These strictly adhere to the ARRIVE guidelines, the UK Animal Law (Scientific Procedures Act, 1986, and associated guidelines), the EU Directive 2010/63/EU for animal experimentation and the avian toxicity testing studies guidelines of the OECD (OECD, 2016).

At five weeks old, the Japanese quails were randomly assigned to four dosage groups ($n=7$ per group). The three treatment groups received different concentrations of IMI (purity $\geq 98.0\%$, Sigma-Aldrich, USA) dissolved in distilled water, at doses of 1.55 mg/kg (low), 3.1 mg/kg (medium), and 6.2 mg/kg (high), while the control group was administered only distilled water (10 mL/kg). The doses were given via intragastric gavage, twice a week, for four weeks. Before dosing, the birds were fasted overnight, and the dose was calculated based on the body weight measured on the previous day before fasting commenced and adjusted to a dosing volume of 10 mL/kg. The doses of

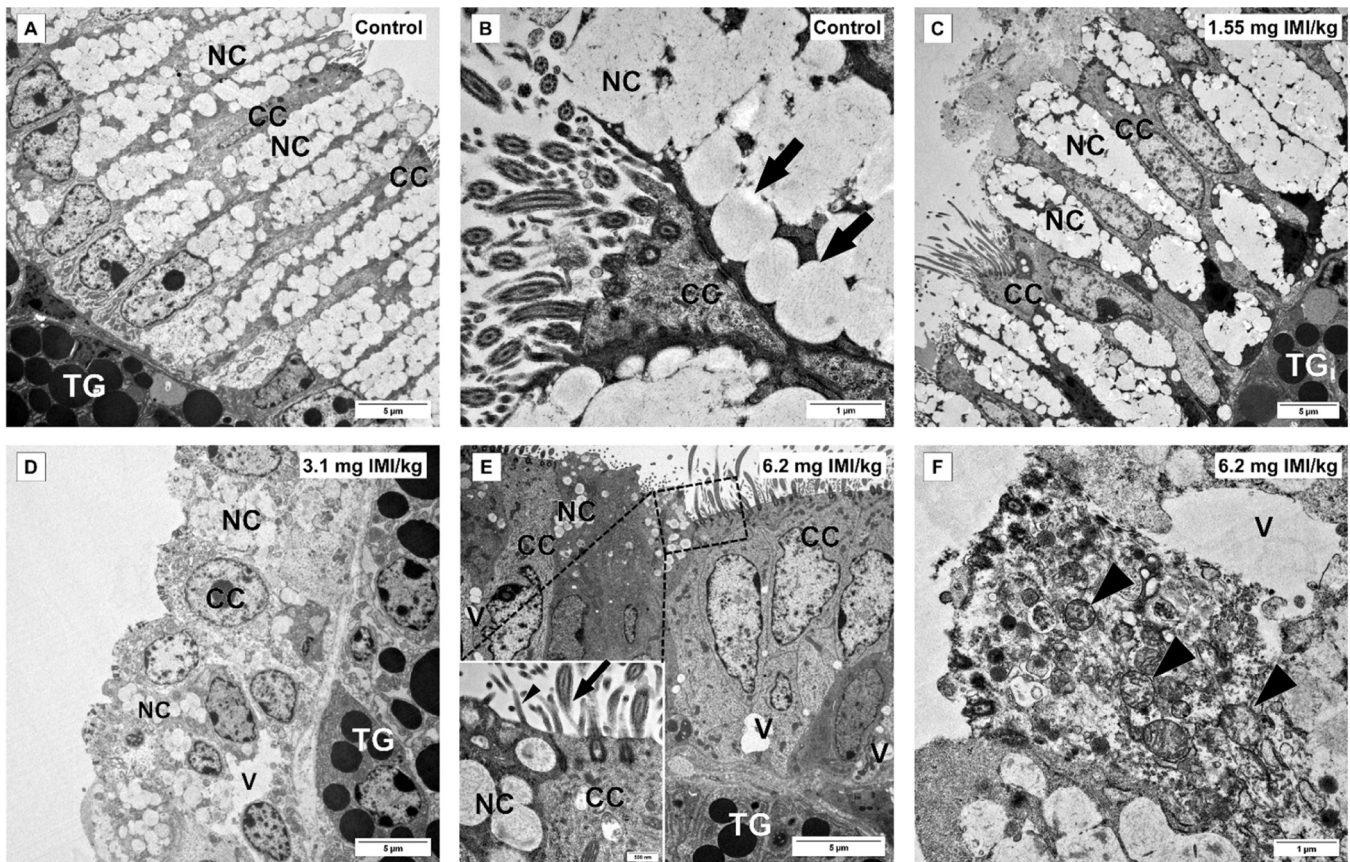


Fig. 3. Transmission electron photomicrographs of the magnum in Japanese quails following imidacloprid (IMI) treatment. (A&B) control, and (C) low (1.55 mg/kg), (D) medium (3.1 mg/kg) and (E&F) high (6.2 mg/kg) IMI-dosage groups. CC: Ciliated cells. NC: Nonciliated cells. V: Cytoplasmic vacuoles. TG: Tubular glands. Thick arrows indicate membrane-bound electron-lucent secretory vesicles in NC. Thick arrowheads indicate mitochondrial damage. Inset: Higher magnification showing cilia (thin arrow) and microvilli (thin arrowheads) on the apical surfaces of CC and NC, respectively.

IMI corresponded to 5 %, 10 %, and 20 % of the median lethal dose in Japanese quail, $LD_{50} = 31$ mg/kg (EFSA, 2014; SERA, 2005).

2.2. Microscopy

At 10 weeks old, the quails were euthanized using the same methods described elsewhere by Ibrahim et al. (2024). Briefly, the birds were individually placed into the euthanasia chamber, containing a gas mixture of N_2 (35 %), CO_2 (35 %), and O_2 (30 %). Thereafter, the abdominal cavity was opened immediately, and the intestinal tract displaced to expose the genital tracts. Tissue samples were obtained from the middle parts of the magnum and shell gland, and the epididymal region (including rete testis, efferent ducts, and epididymal duct).

For light microscopy, tissue samples were immediately immersed in 10 % paraformaldehyde, fixed and routinely processed. Histological Section (5 μ m thick) were stained with hematoxylin, and eosin (H&E) and images (40x) were captured using a digital camera connected to an Olympus BX-63 microscope (Olympus Corporation, Japan). For histometric analysis, the epithelium height (μ m) of the of the magnum, shell gland, and different ducts of the epididymal region were measured using a Fiji-ImageJ software (Schindelin et al., 2012) following the same methods described in our earlier study (Ibrahim et al., 2024). Briefly, the epithelial heights were measured in at least five regions, selected randomly, per tubule of the genital tract regions of male and female quails in the control and treatment groups, and determined from the periductal layer (basal lamina) to the luminal edge. To minimize variability due to sectioning angle, only circular or oval tissue cross sections were selected for analysis.

For electron microscopy, following fixation and post-fixation in

2.5 % glutaraldehyde (pH 7.4) and 1 % osmium tetroxide for 24 h and 2 h, respectively, tissue samples were subsequently processed following the method described by Ibrahim et al. (2021) for transmission electron microscopy (TEM) and by Madekurozwa and Mpango (2020) for scanning electron microscopy (SEM). Micrographs were acquired using a JEOL JEM 1400-FLASH (TEM, JEOL Japan), and a FEGSEM: Zeiss 540 Ultra SEM (Carl Zeiss, Germany).

2.3. Statistical analysis

The data were analyzed using IBM SPSS 28 (IBM Corporate, USA), and tested for homogeneity of variance, descriptive statistics and plotting histograms. Thereafter, data from the control and IMI-treatment groups were analyzed using a one-way analysis of variance (ANOVA) and the significant differences set at $P \leq 0.05$. The data are expressed as mean \pm standard error (SE).

3. Results

Compared to the control birds, there were several structural changes observed in the cells of the genital tract after pubertal exposure to IMI at medium (3.1 mg/kg) and high (6.2 mg/kg) doses, twice a week for 30 days. However, no remarkable pathomorphological changes were detected in birds exposed to IMI at a low dose (1.55 mg/kg).

3.1. Pathomorphology of the magnum

In both control and low IMI dose birds, the magnum epithelial and glandular cells were normally arranged. The epithelium was simple

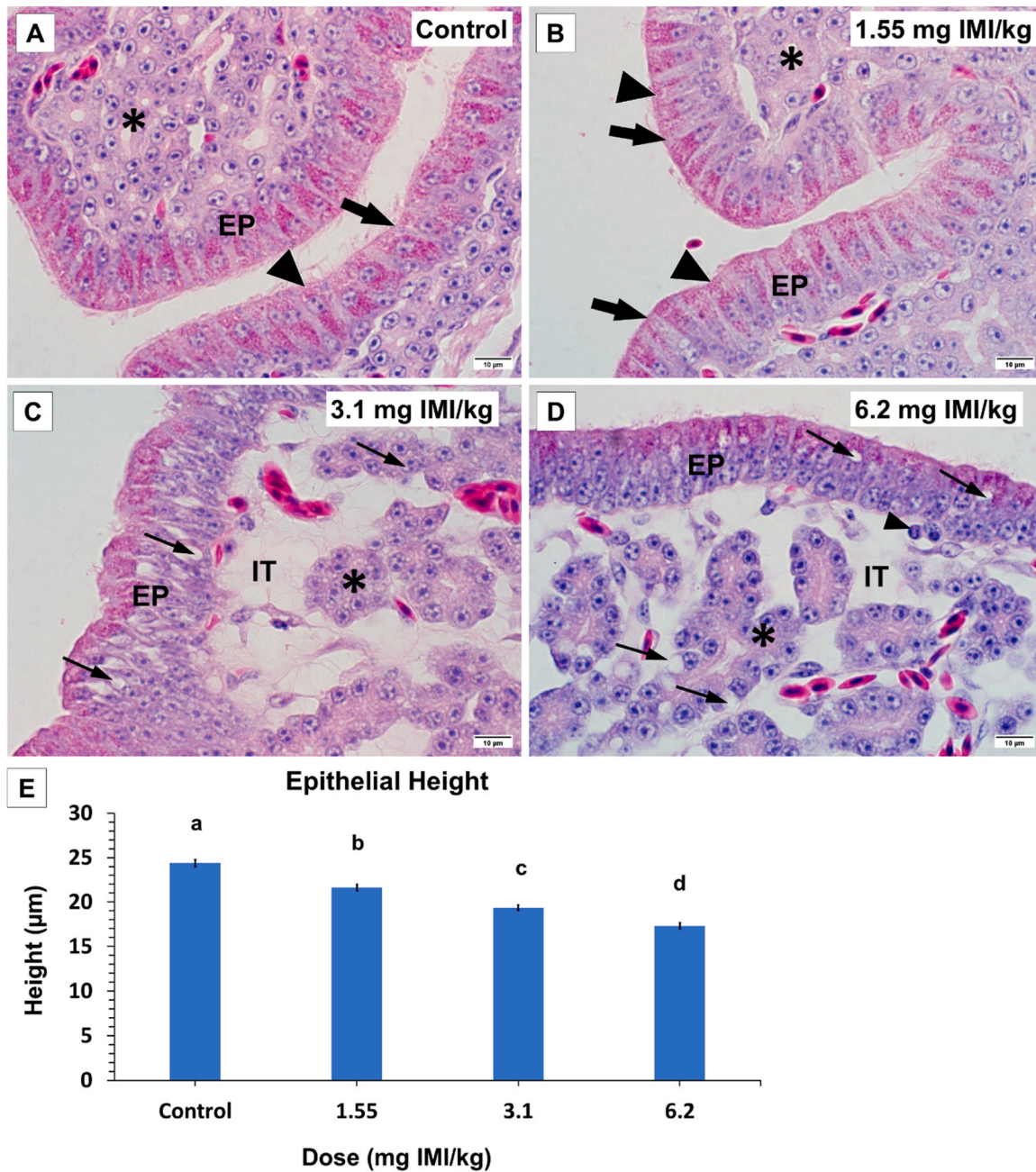


Fig. 4. Light micrographs of shell gland of Japanese quails following imidacloprid (IMI) exposure. (A) control, (B) low (1.55 mg/kg), (C) medium (3.1 mg/kg) and (D) high (6.2 mg/kg) IMI-dosage groups. (A & B) Pseudostratified columnar epithelium (EP) contains ciliated (thick arrowheads) and nonciliated (thick arrows) cells. (C & D) Thin arrows: vacuoles in the epithelial and glandular cells. The gland cell displays a pyknotic nucleus (thin arrowhead). Asterisks: tubular glands. IT: space between tubular glands. (E) Histometric measurements (mean \pm SE) of height of epithelium of the shell gland of the quails ($n = 4$ per group) following treatment with three doses of IMI. Statistical differences ($P \leq 0.05$) between groups are indicated by (a, b, c, d).

columnar and consisted of two distinct type of cells; namely ciliated cells, which contained cilia and microvilli on their apical surface, as well as nonciliated cells only covered by microvilli (Figs. 1A & B, 2A & B, and 3A–C). In addition, the cytoplasm of the nonciliated cells contained abundant secretory vesicles (Fig. 1A & B, 2A&B, and 3A–C). In the medium and high IMI dose, however, the infranuclear regions of the magnum epithelial and glandular cells contained numerous intracytoplasmic vacuoles (Fig. 1C & D, and 3D–F). A few secretory vesicles in the cytoplasm of nonciliated cells, and mitochondrial damage with loss of cristae in the ciliated cells were detected in the birds exposed to the medium and high IMI doses (Fig. 3D–F). In addition, observations of the magnum structure of quails revealed degenerative changes such as

loss of cilia and a few, short microvilli in the nonciliated and ciliated cells in birds treated with the medium and high IMI dose (Fig. 2C & D). In comparison with the control birds, the heights of the magnum epithelia were decreased significantly ($P \leq 0.05$) in three IMI exposure groups (Fig. 4E).

3.2. Pathomorphology of the shell gland

The shell gland epithelium was pseudostratified columnar and consisted of nonciliated and ciliated cells (Figs. 4A–D, 5A & B, and 6A & B). The control birds and those treated with low IMI dose showed numerous eosinophilic granules in the cytoplasm of the nonciliated cells (Fig. 4A,

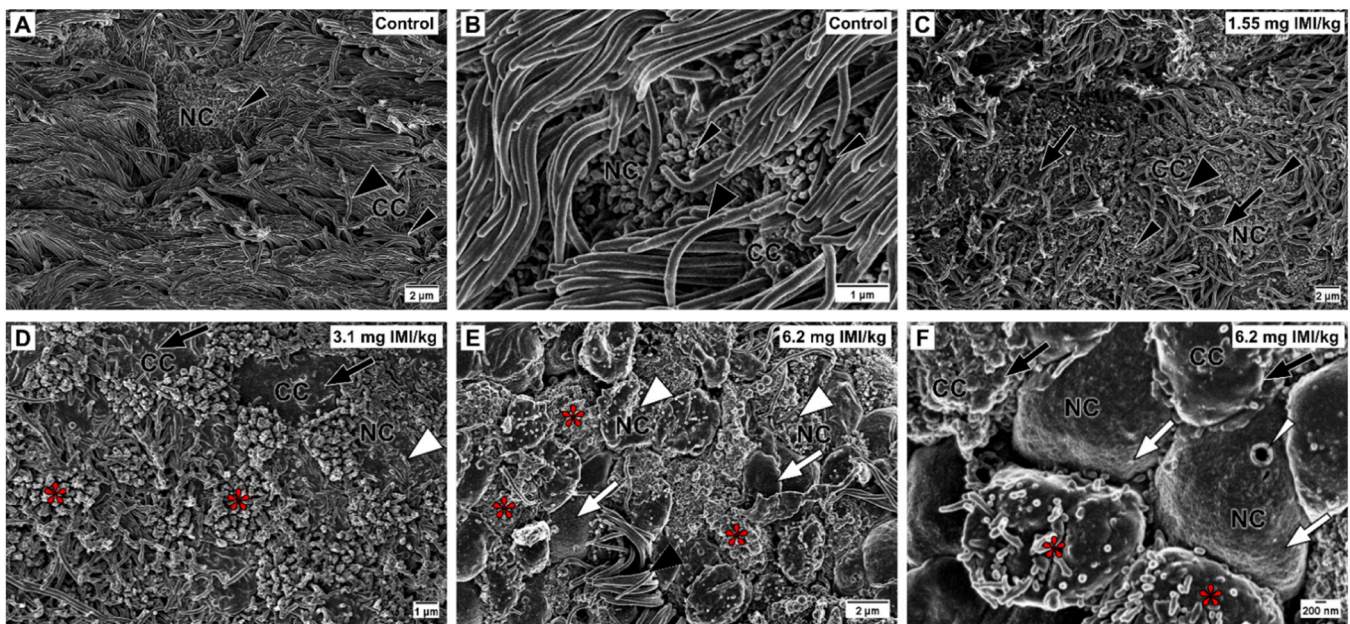


Fig. 5. Scanning electron micrographs of shell gland in Japanese quails following imidacloprid (IMI) treatment. (A&B) control, and (C) low (1.55 mg/kg), (D) medium (3.1 mg/kg) and (E&F) high (6.2 mg/kg) IMI-dosage groups. CC: ciliated cells. NC: nonciliated cells. Thick black arrowheads: cilia. Thin black arrowheads: microvilli. (C) Ciliated cells (black arrows) are covered with a few cilia. (D) Black arrows indicate ciliated cells displaying ciliary loss with remnant cilia. Thick white arrowhead: nonciliated cell with swollen microvilli. Asterisks: ciliated cells covered with microvilli. (E&F) Nonciliated cells with smooth surfaces (white arrows) or displaying few short microvilli (thick white arrowheads). Asterisks: ciliated cells covered with microvilli. Ciliated cells display loss of cilia with remnant cilia (black arrows). Thin white arrow: apical cellular pit.

B). In comparison, in the birds treated with the medium and high IMI dose, fewer eosinophilic granules were present and only in the apical region of these cells (Fig. 4C, D). In addition, we observed an increase in the space between tubular glands, and cytoplasmic vacuoles in the epithelium and glandular region of the shell gland in quails treated with medium and high IMI dose (Fig. 4C, D). A few ciliated cells in shell gland exhibited a partial ciliary loss in their luminal surfaces in quails exposed to the low IMI dose, while in the birds treated with middle and high IMI dose, the nonciliated cells also exhibited short, swollen microvilli (Fig. 5D–F). A total loss of microvilli was also seen in some nonciliated cells of the shell gland in birds treated with high IMI-dose (Fig. 5E & F). Furthermore, TEM analysis revealed that the cytoplasm of the nonciliated and ciliated cells in birds treated with medium-dose IMI had fewer secretory granules and vacuoles. However, an absence of secretory granules, damaged mitochondria, dilated rough endoplasmic reticulum and vacuoles were noticed in the cytoplasm of the cells lining the shell gland in quails treated with high IMI-dose (Fig. 6D, E). In addition, dilatation of the rough endoplasmic reticulum and mitochondrial damage was also seen in the tubular gland cells in birds treated with medium and high IMI-dose (Fig. 6F). Histometrically, in comparison with the control group, the heights of the shell gland epithelia were reduced significantly ($P \leq 0.05$) in all three IMI exposure groups (Fig. 4E).

3.3. Pathomorphology of the epididymal region

The epididymal region consists of rete testis, efferent ducts (proximal and distal segments) and epididymal duct.

3.3.1. Rete testis

Compared to the control group, the rete testis epithelium did not show any obvious pathomorphological changes in the three IMI exposure groups (Fig. 7A–F). The rete testis epithelium consisted of simple squamous or cuboidal cells. A few short microvilli were detected in the apical surfaces of the cuboidal cells, while the cytoplasm of these cells contained a few organelles, including rough endoplasmic reticula and

mitochondria (Fig. 7D–F). However, the height of the rete testis epithelium was decreased significantly ($P \leq 0.05$) in all treated groups, when compared with those of the control group (Table 1).

3.3.2. Efferent ducts

The efferent ducts consist of proximal and distal segments. The luminal epithelium of both segments were lined with ciliated and nonciliated cells (Figs. 8A–D, 9A–E, and 10A–D). In all treated groups, intracytoplasmic vacuoles were detected in the nonciliated and ciliated cells of the proximal efferent ducts (Figs. 8A–D and 9A–F). In addition, in the birds treated with high IMI doses, autolysosomes and degenerated secretory granules with lipid droplets were detected in the proximal efferent duct epithelium (Fig. 9E, F).

In the distal efferent ducts, there were no ultrastructural changes observed in the nonciliated and ciliated cells of quails exposed to the low IMI dose; however, intracytoplasmic vacuoles were seen in cells lining the distal efferent ducts in birds treated with medium and high IMI doses (Fig. 10A–D).

In comparison with the control group, the epithelial heights of the proximal segment of the efferent ducts decreased significantly ($P \leq 0.05$) in all three IMI exposure groups as the dose rate increased (Table 1). In the distal efferent ducts, there were no differences in the epithelial height between birds treated with low and medium IMI-dose, while the height of epithelium was decreased significantly ($P \leq 0.05$) in birds exposed to high IMI-dose (Table 1).

3.3.3. Epididymal duct

The epithelium of the epididymal duct consisted of nonciliated columnar and basal epithelial cells (Fig. 11A–C). In birds exposed to the low IMI dose compared with the control group, IMI did not cause any observable structural changes in the cells lining the epididymal duct. However, numerous, large intracytoplasmic vacuoles were detected in the nonciliated columnar cells of this region in quails exposed to medium and high IMI doses (Fig. 11B, C, E, F). In addition, there were no differences in the epithelial height of the epididymal duct in birds exposed to low IMI dose and the birds of the control group, while the

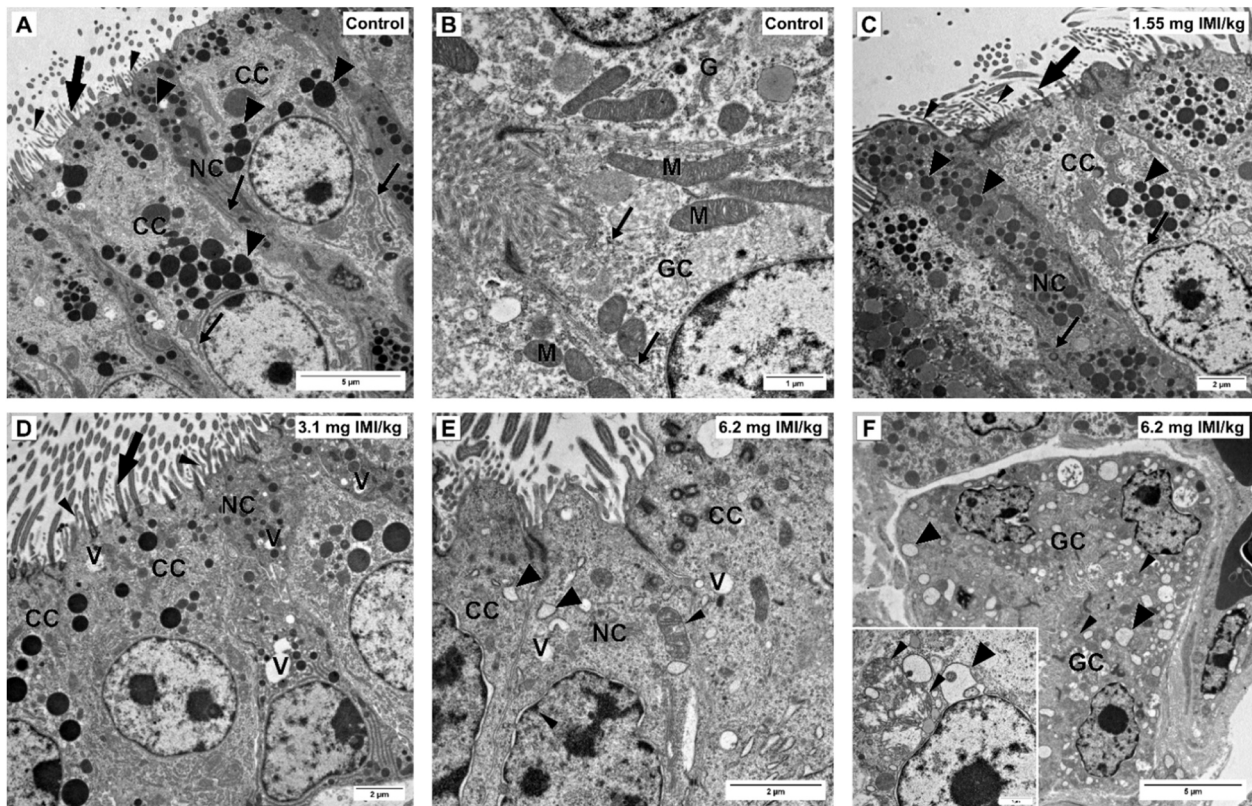


Fig. 6. Transmission electron micrographs of the shell gland in Japanese quails following imidacloprid (IMI) treatment. (A&B) control, and (C) low (1.55 mg/kg), (D) medium (3.1 mg/kg) and (E&F) high (6.2 mg/kg) IMI-dosage groups. (A, C & D) Microvilli (thin arrowheads) line the luminal surface of ciliated (CC) and nonciliated (NC) cells, and cilia (thick arrows) line ciliated cells. (A & C) Nonciliated and ciliated cells display secretory granules (thick arrowheads) and rough endoplasmic reticulum (thin arrows). (B) Tubular gland cells (GC) display normal mitochondria (M), Golgi complex (G), and rough endoplasmic reticulum (thin arrows). (D) The cytoplasm of nonciliated and ciliated cells contains few secretory granules and displays large cytoplasmic vacuoles (V). (E) The cytoplasm of nonciliated and ciliated cells lacks secretory granules and contains damaged mitochondria (thin arrowheads). (F & inset) Tubular gland cells (GC) exhibit damaged mitochondria (thin arrowheads) and dilated rough endoplasmic reticulum (thick arrowheads).

epithelial heights in quails exposed to medium and high IMI doses decreased significantly ($P \leq 0.05$) (Table 1).

4. Discussion

The current study evaluated the effect of various doses of the neonicotinoid pesticide, imidacloprid (IMI), on the structure of female and male genital tracts, including the magnum, shell gland, and epididymis of Japanese quail (*Coturnix Coturnix japonica*). Several morphological changes were observed in the genital tract sections of the male and female quails following exposure to 1.55 (low), 3.1 (medium), and 6.2 (high) mg/kg IMI from 5 to 9 weeks post-hatching, with changes appearing to occur in a dose-dependent manner. To our knowledge, this is the first report of the deleterious toxic effects of IMI on the genital tracts of birds. In previous studies, IMI reproductive toxicity was assessed by means of parameters such as body condition, oxidative stress, the first egg-laid date, clutch size, and egg yolk vitamins in the red-legged partridge (Lopez-Antia et al., 2015), and body weight, and testis histopathology in red munia (Mohanty et al., 2017). Furthermore, imidacloprid-induced hepatotoxicity, neurotoxicity, oxidative stress, and lipid peroxidation have been confirmed in Japanese quail. (Osman et al., 2023).

There were more structural changes in the magnum and shell gland of the birds exposed to medium and high IMI doses than those treated with the low IMI dose. The histometric measurements indicated that IMI decreased the epithelial height of the magnum and shell gland in all

treated birds. However, it should be noted that the histometric measurements in this study were performed using a two-dimensional (2D) cross-section of the genital tract regions, which represent only a slice of the complete three-dimensional (3D) structure. It has also been reported that IMI induces a height reduction in the thyroid follicular epithelium in wild birds (Pandey and Mohanty, 2015), which could contribute to its endocrine-disrupting activity (Mikolić and Karačonji, 2018). The reduction in the epithelial heights of the magnum and shell gland, induced by the fungicide, carbendazim, is attributed to oviductal atrophy due to the endocrine disruptive effects of the fungicide in Japanese quails (Kimaro, 2014; Kimaro et al., 2013).

Furthermore, degenerative changes such as cytoplasmic vacuoles, mitochondrial damage, and fewer secretory granules were detected in the cells of the magnum and shell gland of Japanese quails treated with medium and high IMI doses in this study. Lohiya et al. (2019) reported that IMI causes mild vacuolation in the epithelium of the uterus in rats after treatment with 38 mg/kg IMI. Mitochondrial damage and cytoplasmic vacuoles in the rat Leydig cell line (LC-540) (Ibrahim et al., 2023), and in the brain and liver of the rock pigeon (Abu Zeid et al., 2019), were also observed following exposure to IMI. Any pathological changes in the structure of the magnum could result in thin and watery albumen due to decreased albumen synthesis (Chousalkar and Roberts, 2007), while alterations in the shell gland structure are associated with poor eggshell quality (Cheng and Ning, 2023). Thus, histomorphological changes observed in the magnum and shell gland tissue may affect egg production and quality in these birds, ultimately resulting in infertility.

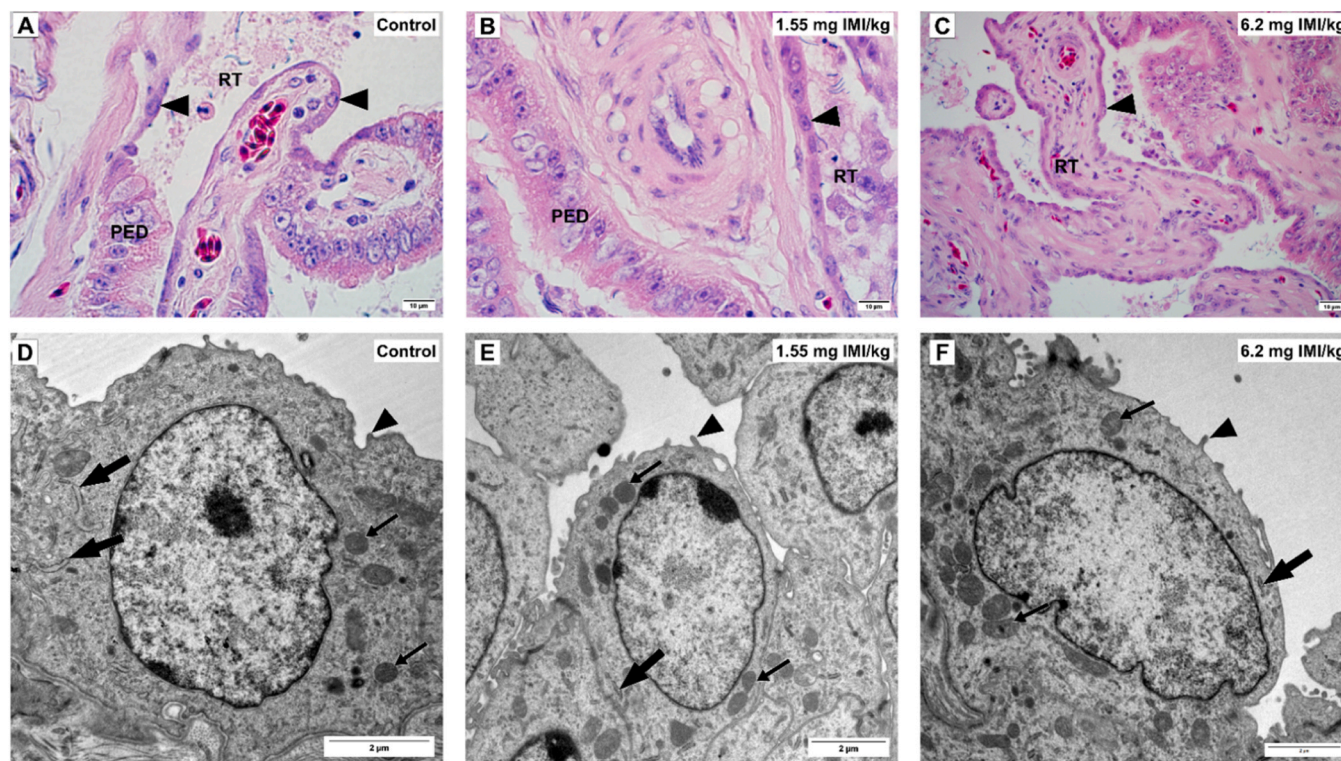


Fig. 7. Photomicrographs of rete testis epithelium in Japanese quails following imidacloprid (IMI) treatment. (A&D) control, and (B&E) low (1.55 mg/kg), and (C&F) high (6.2 mg/kg) IMI-dosage groups (A–B) Arrowheads: simple squamous or cuboidal epithelium lining the rete testis (RT). (D–F) Arrowheads: Microvilli. Thin arrows: Mitochondria. Thick arrows: Rough endoplasmic reticulum. PED: Proximal efferent duct.

Table 1

The heights of epithelium (μm) of the epididymal region of the Japanese quail exposed during puberty to imidacloprid (IMI) at 1.55 mg/kg (low), 3.1 mg/kg (medium) and 6.2 mg/kg (high) twice per week for 30 days.

Parameters	Experimental Groups ($n = 3$)			
	Control	Low IMI-dose	Medium IMI-dose	High IMI-dose
Rete testis (μm)	6.87 ± 0.41^a	5.26 ± 0.47^b	4.40 ± 0.39^c	3.68 ± 0.38^d
Proximal efferent duct (μm)	21.82 ± 0.69^a	17.44 ± 0.67^b	15.14 ± 0.99^c	12.77 ± 0.99^d
Distal efferent duct (μm)	11.58 ± 0.41^a	11.23 ± 0.66^a	10.96 ± 0.72^a	9.27 ± 0.89^a
Epididymal duct (μm)	17.26 ± 0.54^a	16.11 ± 0.88^a	14.28 ± 0.94^b	11.73 ± 1.03^c

Note: Means \pm SE with different superscripts (^{a,b,c,d}) within rows differ significantly between groups ($p \leq 0.05$). Low IMI-dose: 1.55 mg/kg. Medium IMI-dose: 3.1 mg/kg. High IMI-dose: 6.2 mg/kg.

Sabin and Mora (2022), reported that IMI causes eggshell thinning as well as reduced clutch size, egg viability and egg hatchability in bobwhite quails.

In the current study, scanning electron microscopy revealed total or partial loss of cilia on the luminal surfaces of the magnum, and shell gland in birds exposed to medium and high IMI doses, twice per week during puberty. It has been demonstrated that cytoskeletal proteins such as actin microfilaments and microtubules are essential for the development and maintenance of cilia (Ge et al., 2022). Exposure to imidacloprid induces alterations in the structure of β -tubulin microtubules and F-actin microfilaments in the Leydig cell line (LC-540) (Ibrahim et al., 2023). Therefore, the loss of cilia in the magnum and shell gland observed in this study might be due to IMI-induced cytoskeletal disruption. Cilia transport spermatozoa through the female genital tract (Johnson, 2015), and ciliary loss may affect fertility.

The male genital tract plays a crucial role in spermatozoal transit and enhances the fertilization capacity of spermatozoa (Jiménez-Reina et al., 2016). In the present study, exposure of quails to IMI during puberty altered the morphology of the epithelial cells in the epididymal region.

The effects of this neonicotinoid were dose-dependent, suggesting that it may disrupt the function of the male genital tract in birds. In the proximal efferent ducts, major structural alterations after treatment with IMI were observed. These changes included numerous cytoplasmic vacuoles, autolysosomes and degenerated secretory granules with lipid droplets. As the efferent ducts constitute approximately 56 % of the total epididymal region in Japanese quail (Aire, 1979), and are responsible for reabsorbing over 86 % of the fluid released from testis (Clulow et al., 1994; Clulow and Jones, 1988) the morphological changes observed in this study could result in decreased semen quality.

The sensitivity of the proximal efferent ducts to endocrine-disrupting chemicals has previously been reported in Japanese quails treated with di-*n*-butyl phthalate (Ibrahim et al., 2021), and diethylstilbesterol (Yoshimura and Kawai, 2002), and in drakes treated with glyphosate (Oliveira et al., 2007). In addition, efferent ducts are a major estrogen-dependent site in the genital tract of males (Hess and Carnes, 2018). Due to the reported antiestrogenic activity of IMI (Zhang et al., 2020), it is possible to surmise that IMI also alters the estrogenic functions in the efferent ducts of Japanese quail.

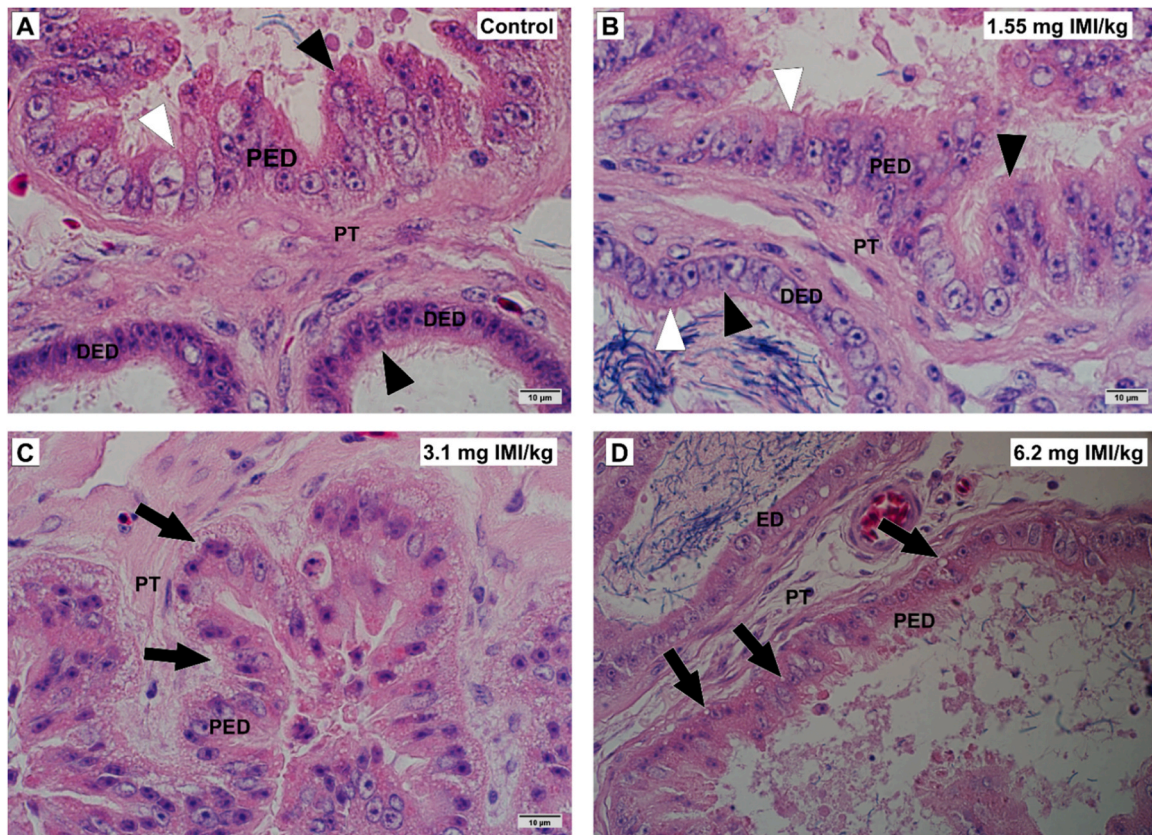


Fig. 8. Photomicrographs of proximal (PED) and distal (DED) efferent ducts luminal epithelia in Japanese quails following imidacloprid (IMI) treatment. (A) control, (B) low (1.55 mg/kg), (C) medium (3.1 mg/kg) and (D) high (6.2 mg/kg) IMI-dosage groups. Black arrowheads: Nonciliated cells. White arrowheads: Ciliated cells. Black arrows: Cytoplasmic vacuoles. PT: Peritubular tissue.

The present study has some limitations that should be acknowledged. We studied the effects of oral administration of IMI on the genital tracts of male and female quails, but IMI could also influence other physiological systems and processes. Imidacloprid has been reported to induce hepatotoxicity (Emam et al., 2018; Osman et al., 2023), neurotoxicity (Rawi et al., 2019), and oxidative stress (Osman et al., 2023) in Japanese quail, suggesting that its systemic effects may potentially indirectly contribute to pathomorphological changes detected in the genital tracts. Therefore, future studies are warranted to explore the relationship between systemic toxicity of IMI and the reproductive system.

5. Conclusion

This study reports, for the first time, that exposure of Japanese quail (*Coturnix Coturnix japonica*) to imidacloprid (IMI) during puberty, may cause pathomorphological changes in the genital tracts of male and female quails. Imidacloprid-induced degenerative changes included a decrease in epithelial height, ciliary loss, cytoplasmic vacuoles, mitochondrial damage, and fewer secretory granules in the magnum and shell gland. In addition, intracytoplasmic vacuoles, autolysosomes and degenerated secretory granules with lipid droplets were detected in the proximal efferent duct epithelium. In general, IMI caused more damage to the female genital tract of Japanese quails than their male counterparts.

The results of this study highlight important implications for wild bird populations, particularly those that may ingest IMI-treated seeds in agricultural settings (Elbert et al., 2008). The observed structural alterations in genital tracts of both male and female quails suggest that IMI exposure disrupts epithelial integrity and cellular metabolism. These effects indicate potential mechanisms of toxicity involving mitochondrial dysfunction and impaired secretory and absorptive functions,

which could contribute to a decline in bird populations through impaired fertility and reduced egg production and quality. Further investigations are required to explore potential preventative strategies for avian species inhabiting IMI-contaminated environments, as well as to consider appropriate measures to restrict IMI use in ecologically sensitive areas.

Author statement

All authors affirm that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. In addition, authors have confirmed that the manuscript has been read and approved by all named authors and that there are no other persons who satisfied the criteria for authorship but are not listed. Therefore, there is no conflict of interest.

Authors understand that the Corresponding Author is the sole contact for the Editorial process. He is responsible for communicating with the other authors about progress, submissions of revisions and final approval of proofs.

CRediT authorship contribution statement

Rephima M. Phaswane: Writing – review & editing, Visualization, Validation, Methodology. **Ibrahim Mohammed Ibrahim Ahmed:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Conceptualization. **Christo J. Botha:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Data curation, Conceptualization. **Antoinette V. Lensink:** Writing – review & editing, Visualization, Validation, Methodology.

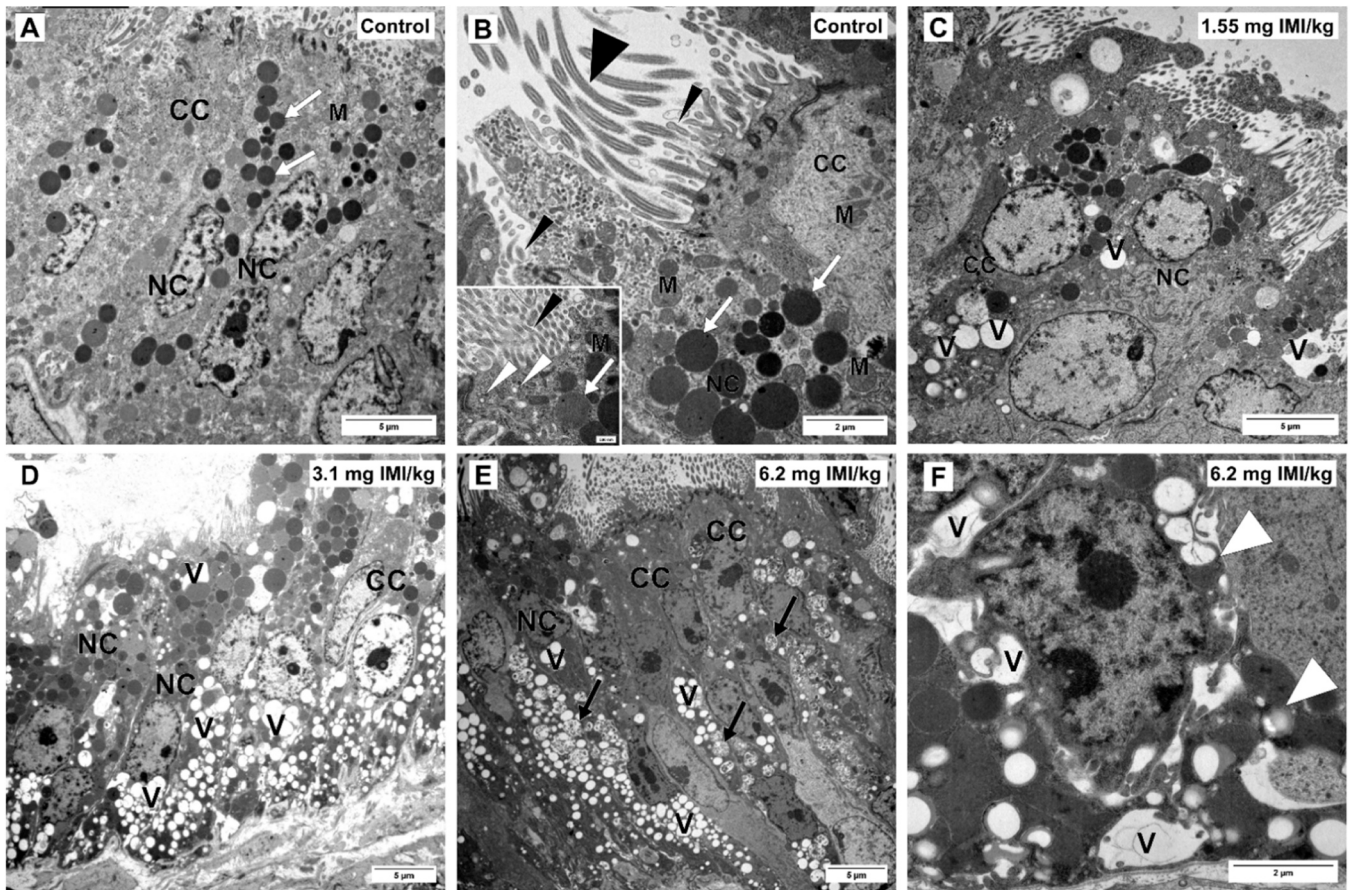


Fig. 9. Photomicrographs of the proximal efferent duct in Japanese quails following imidacloprid (IMI) exposure. (A&B) control, and (C) low (1.55 mg/kg), (D) medium (3.1 mg/kg) (E&F) high (6.2 mg/kg) IMI-dosage groups. (A&B) Microvilli (black thin arrowheads) line the luminal surface of ciliated (CC) and nonciliated (NC) cells. Cilia (black thick arrowhead) of the ciliated cell. Nonciliated cells exhibiting secretory granules (white arrows). Inset: Higher magnification showing microvilli (black thin arrowhead), coated apical tubules (white thin arrowheads), and secretory granules (white arrow) are observed in the subapical cytoplasm of the NC Type in the proximal efferent duct. (C-F) Cytoplasmic vacuoles (V) in the nonciliated and ciliated cells. (E) Autolysosomes (black arrows). (F) Degenerated secretory granules with lipid droplets (white arrowheads). M: Mitochondria.

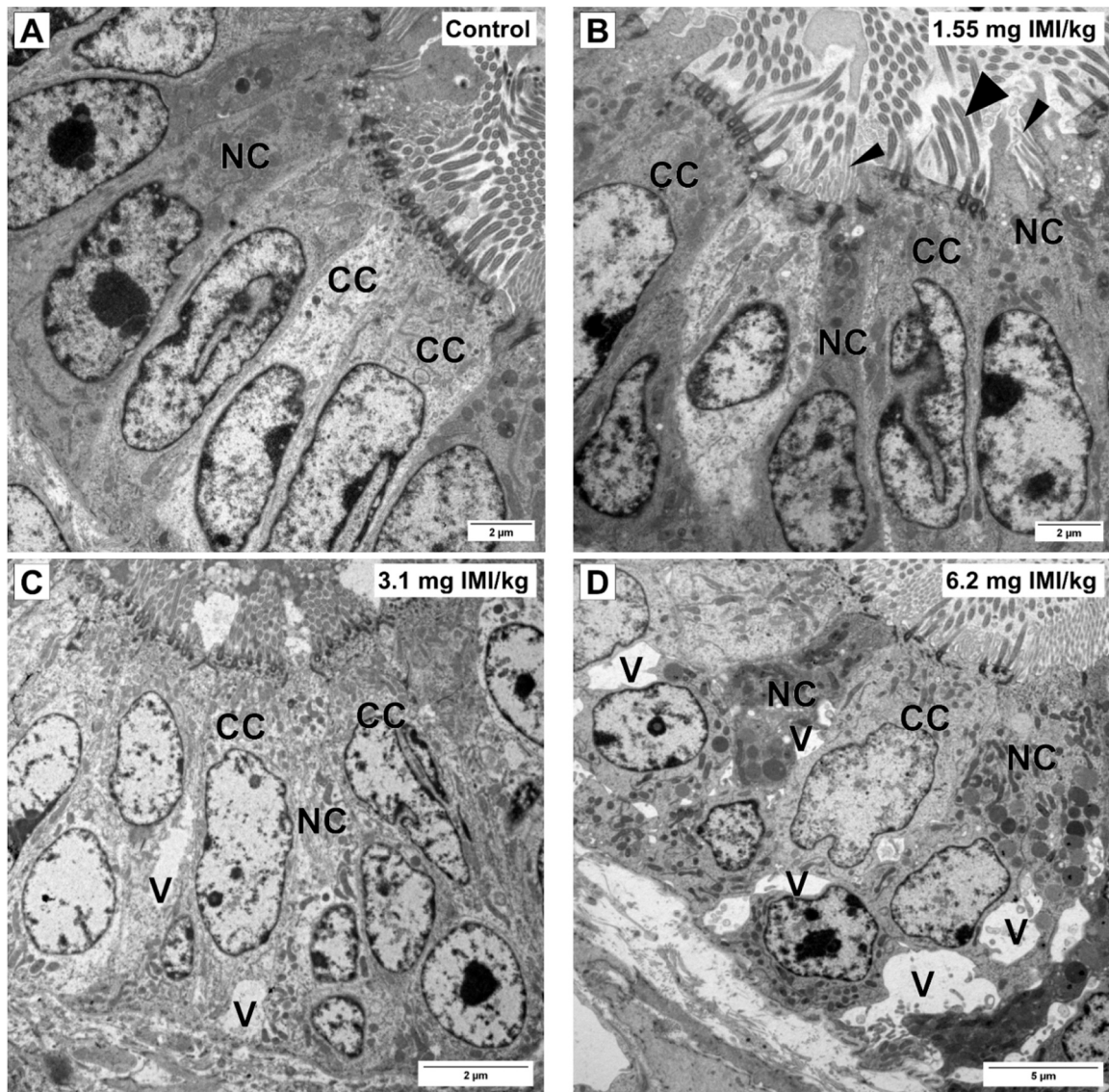


Fig. 10. Photomicrographs of the distal efferent duct in Japanese quails following imidacloprid (IMI) treatment. (A) control, and (B) low (1.55 mg/kg), (C) medium (3.1 mg/kg) and (D) high (6.2 mg/kg) IMI-dosage groups. (A & B) Microvilli (thin arrowheads) line the luminal surface of ciliated (CC) and nonciliated (NC) cells. Thick arrowhead: cilia of the ciliated cell. (C & D) Large cytoplasmic vacuoles (V) in the nonciliated and ciliated cells.

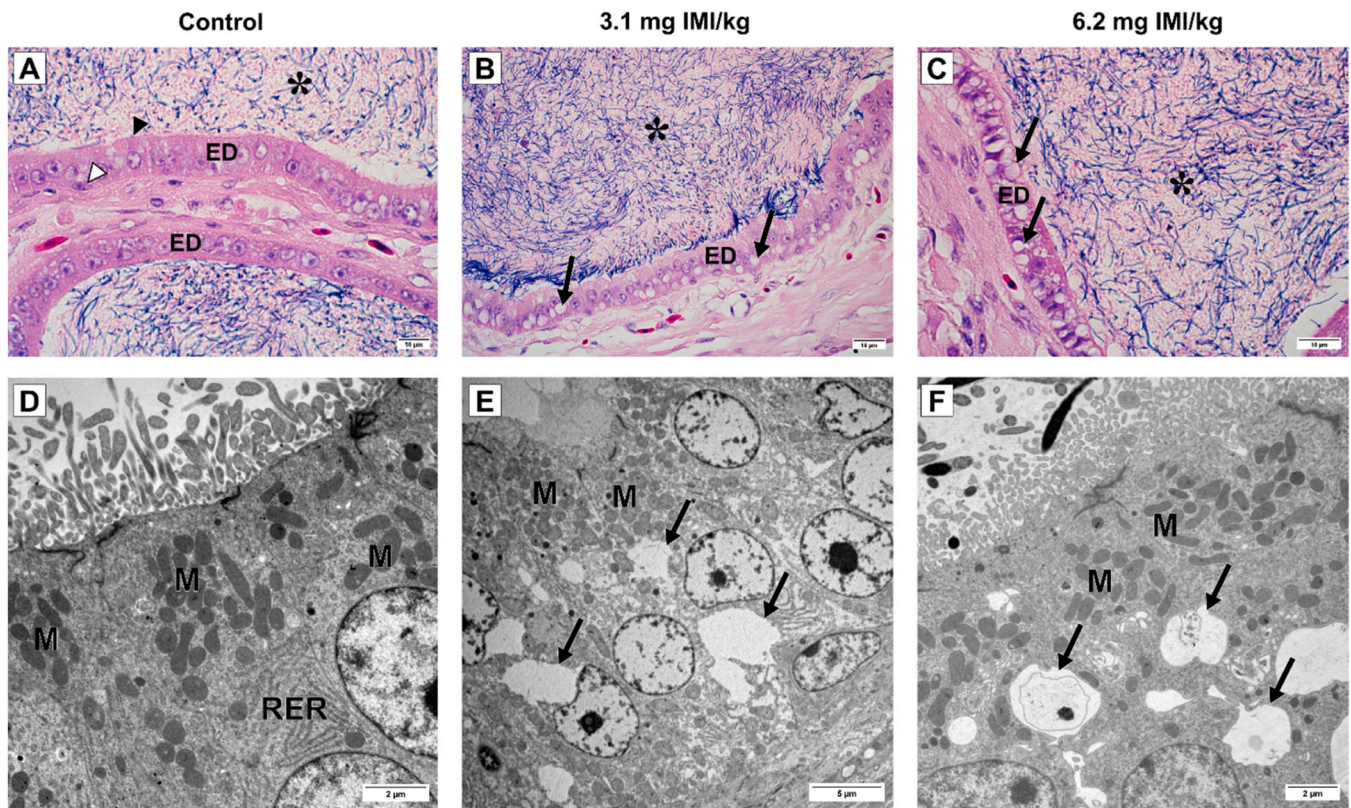


Fig. 11. Photomicrographs of epididymal duct of Japanese quails following imidacloprid (IMI) treatment. (A&D) control, and (B&E) medium (3.1 mg/kg) and (C&F) high (6.2 mg/kg) IMI-dosage groups. (A) Epididymal duct epithelium (ED) consists of nonciliated columnar (black arrowhead) and basal (white arrowhead) cells. (B, C, E & F) Black arrows: Large cytoplasmic vacuoles. M: Mitochondria. RER: Rough endoplasmic reticulum.

Conflicts of Interest

The authors declare no conflict of interest.

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Data availability

Data will be made available on request.

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