International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2025.113.130

Research Article
Pharmacokinetic and Therapeutic Dose Comparison of Diane-35 and IVF-C Inj 5000 IU in Common Quail

[1]Zain Ul Abideen, [2]Mohsen A. Khormi, [2]Sultan M. Areshi, [3]Mehwish Raza, [3]Muhammad Nadeem, [3]Muhammad Amjad, [3]Muhammad Saif Ullah, [1]Aleem Ahmed Khan, [4]Mukul Sharma and [2]A. El-Shabasy

[1]Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan
[2]Department of Biology, College of Science, Jazan University, P.O. Box 114, Jazan 45142, Kingdom of Saudi Arabia
[3]Department of Zoology, Ghazi University, Dera Ghazi Khan, Pakistan
[4]Environment and Nature Research Centre, Jazan University, Jazan 45142, P.O.Box 114, Saudi Arabia

Abstract

Background and Objective: The use of steroids is regarded as the most argued thorny topic due to mutual positive and negative obtained results. This study examines the pharmaco-histokinetic effects on common quail of two different steroids: IVF-C Inj (containing 5000 IU of human chorionic gonadotropin) and Diane-35 (containing cyproterone acetate-ethinyl estradiol). **Materials and Methods:** The 120 common quails were split into winter and summer groups for a two-phase *in vivo* experiment that lasted 10-20 days each. There were 40 treated birds and 20 control birds in each study group, which was then split into two subgroups of 20 birds each. Daily doses of IVF-C Inj (5 IU/kg) and Diane-35 (1 mg/kg) were given to the treatment subgroups. For comparison, clinical symptoms and histological alterations were noted and documented. Correlation analysis besides Simple Linear Regression among co-variant data was performed. **Results:** Hair loss, appetite loss, testicular enlargement, depression and elevated body temperature were significant clinical symptoms seen in all treated birds ($p<0.01$). One and a half percent of the birds who received cyproterone acetate or human chorionic gonadotropin died. According to histological investigation, the heart and skeletal muscle tissues of the majority of treated birds did not change significantly from controls. However, some organ changes, most notably liver failure and testicular darkening, were linked to cyproterone acetate treatment. **Conclusion:** The results show that both steroids, especially cyproterone acetate, have important physiological effects on common quail. The need for caution while using steroids is highlighted by observed negative effects on vital organs. These investigations also can differentiate between actual and simulated animals using pharmaco-histokinetic markers.

Key words: Steroids, testis, histology, morphology, therapeutic dose, pharmaco-histogenetic markers, clinical symptoms

Citation: Zain Ul Abideen, M.A. Khormi, S.M. Areshi, M. Raza and M. Nadeem *et al.*, 2025. Pharmacokinetic and therapeutic dose comparison of Diane-35 and IVF-C Inj 5000 IU in common quail. Int. J. Pharmacol., 21: 113-130.

Corresponding Author: Zain Ul Abideen, Institute of Zoology, Bahauddin Zakariya University, Multan, Pakistan

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Transport proteins are involved in steroidogenesis, changing cholesterol into steroid hormones[1], enzymes, redox allies and cofactors[2,3]. The majority of steroidogenic enzymes are either hydroxysteroid dehydrogenases or variants of cytochrome P450[4]. Because they resemble domestic chickens in both anatomy and physiology and are relatively small, common quails are excellent models for studying poultry[5]. The primary benefit of utilizing this species as a model animal for birds is its modest size. When fully grown, the males weigh 100-120 g and the females 120-150 g[6]. Their high egg production rate (up to 300 per year) and short generation interval make them easy to manage in a lab setting. Because quails are sensitive to environmental changes, they are housed in ideal conditions[7].

Strong chemicals called steroids affect nearly every cell in an organism, including the neurological system in both vertebrates and invertebrates[8]. Some of the most widely used medications for birth control, hormone replacement treatment and other purposes are steroids, both natural and synthetic. Although steroids are widely used, there is still much to learn about how they affect various organs[9]. Pharmacokinetics describes the absorption, metabolism and excretion of drugs from the body[10,11].

Within a few days, compounds and metabolites are eliminated from the body through urine and feces. Drug absorption is also dependent on the length of the fatty acid chain; the stronger the absorption, the shorter the chain and vice versa. Metabolism of anabolic steroids mostly takes place in the liver. Anabolic steroids are quickly eliminated from the body since their rate of dispensation is nearly equal to that of introduction. When comparing kidneys, feces and urine, the residual anabolic agent concentration in muscles is the lowest[12].

The easiest way to understand how locally and peripherally produced steroids affect brain functions, including behavior, is to look at bird research[13]. The brains of quail (*Coturnix japonica*) and zebra finch (*Taeniopygia guttata*) are capable of de novo steroidogenesis[14]. The avian brain was found to include active P450scc, 3β-HSD, CYP17, 17β-HSD and aromatase enzymes[15]. This results in the generation of several neurosteroids, including testosterone, estradiol, progesterone and androstenedione, from cholesterol[4].

The increased output of songs in nesting birds is proof that steroids secreted by the gonads and transferred to the brain can synchronize behavioral and neurological activity with reproductive physiology[16].

For women with PCOS, Diane-35 is a reliable drug[17]. It works by blocking the effects of androgens like testosterone and activating the progesterone receptor[18]. Furthermore, it raises serum sex hormone binding globulin SHBG produced by the liver, which lowers free T[19]. During the C-IVF treatment process, large hormone dosages are used to stimulate the ovaries to create more oocytes and embryos that are ready for uterine transfer[20].

Corticosteroids cause body fat to accumulate, gastrointestinal transit to slow down, growth and body weight to decrease and elevated plasma glucose levels and increased food consumption due to elevated muscle protein catabolism[21], liver lipogenesis, as well as improve tissue glycogen and lipolysis[22]. Direct action on target tissues is possible with corticosteroids or their effects can be mediated through interactions with other hormones such prolactin, growth hormone, norepinephrine and thyroid hormone[23].

The steroids also show responses in different environments and seasons. Testosterone (T) and other gonadal hormones are one element affecting birds' seasonal investment in their immune systems. However, T has different roles in the immune systems of birds: While some studies found that T could cause immunosuppression, other studies found that humoral or cell-mediated immunity was unaffected by T[24].

Early in development, progesterone and estrogen levels were increased in frog eggs submerged in a solution containing both hormones[25,26], but by the time the eggs reached adulthood, the levels had decreased and were comparable to those in unmodified eggs. Pregnenolone and progesterone can be converted into steroids and treated eggs displayed a short increase in these quantities. The findings indicate that generally speaking, the steroids present in frog eggs are similar to those found in other species of vertebrates that lay eggs. The dynamic nature of steroid levels during development further suggests that growing embryos control their exposure to maternal steroids. The foundation for examining the origins and effects of maternal steroid actions in frogs has been established by these findings[25].

To sum up, the correlation analysis is the first to identify potential antagonistic and synergistic interactions between proteins, eggshell PROTO IX coloring and maternally generated albumen steroids[27]. These relationships are probably the outcome of both passive and compensatory allocation processes. Despite the paucity of studies on the subject, one may wonder if albumen hormones can have any effect at all on growing embryos. About the involvement of these albumen steroids as passive or compensatory maternal effects, we can only make conjectures[28].

There were notable differences in the way that non-obese and obese people used corticosteroids. It has been demonstrated that obese persons who use corticosteroids gain 10.5% greater body weight. A female's cloacal glands shrivel and she loses her ability to copulate when she takes testosterone[29].

Birds have a clear therapeutic use in medicine since they seem to be particularly sensitive to the potential side effects of corticosteroids[30].

Because corticosteroid adverse effects can occur, it is important to use these drugs with caution and at lower dosages than those prescribed for mammals or birds. The proper dosages of different corticosteroids have not been established for every species of bird[31]. Major negative effects are usually less common with shorter-acting, fast-acting drugs than with longer-acting steroids. Therefore, when treating birds, shorter-acting drugs are usually recommended[32].

This study aimed to evaluate the effects of Diane-35 and IVF-C Inj 5000 IU in common quail by studying different parameters like examination of behavior, mortality rate, body weight, patho-morphological observations on heart, muscles, liver and testis besides histopathological findings to stand on the degree of significant impact of steroids.

MATERIALS AND METHODS

Collection of experimental birds: This study was carried out from October, 2023 to July, 2024; the total sum of active quails was one hundred and twenty, earned from the Pakistani Chowk quail market (Dera Ghazi Khan). This season was regarded as the best time to collect the birds due to migration. The body weight of flying creatures ranged from 70-100 g at purchasing time. Birds subjected to Ghazi University under laboratory conditions were kept under keen observation for about 2-3 weeks. Stainless steel cages equipped with wires were manufactured with suitable areas to capture a definite number of birds without any patterns of fighting or competition, then earned to keep individual birds under it. Well-oxygenated, well-cleaned and well-lighted rooms were selected to put up the cages. Before the experiment's initiation, birds were checked attentively and daily food intake was maintained. Thoroughly bird's examination occurred. They served daily with natural clean water and fresh food. The members of each group were differentiated.

Steroid dose and experimental proposal: Two parallel experiments were achieved. One tested group covered the time of about 10 days and another group stretched for about 20 days. Forty birds were put up as untreated individuals, twenty were for separate medication. Resting tested group contained 80 members divided into four groups (two for Diane-35, two for injection) which were further divided into subgroups having 5 members. Drug subjected to experimental birds. Weight-dependent medication was applied.

Steroids of 2 kinds were purchased from Insta Care for pharmaceutical purposes Co., at 149 B Main Broadway, DHA Phase 8, Lahore, for utilization in this project. Four sub-groups were given steroids for 10 days and the other four sub-groups were given steroids for 20 days. Diane-35 was orally given to 4 sub-groups having members (n = 5) once a day for 10 days and the other 4 subgroups were given a tablet dose for 20 days, the injection was given after 5 days to sub-groups in the same manner. The birds' rectal temperatures were checked frequently and they were also weighed every four days until they were dissected. Birds were closely observed to identify any clinical symptoms.

Drugs are implicated according to their body weight.

Drug dosage rate:

- Diane-35 (1 kg^{-1})
- IVF-C Inj 5000 IU (5 IU per bird)

Examination of behavior: To inspect variations in activity method, birds explored 3 times in a day, bleeding from nasal passage area marked as they might squabble. Feed reduction, uneasiness, depression, annoyance behavior and excitement to feed, body weight variations, body temperature changes and drowsiness were observed. Vivisection of those birds occurred immediately; they died throughout the project.

Parameters for morphological-histological reasoning: Treated and untreated aggregations then vivisected at the 4th, 7th, 10th, 13th, 16th and 19th days to assemble certain vital organs heart muscles, kidneys, skeletal muscles and liver as shown in Fig. 1.

The dissected bird's organs were gathered for histological examination. The dissected bird's vital organs were put up immediately in a saline solution.

Tissue processing and microtomy: Tissue processing is done by carrying out Grossing, Fixation, Dehydration and Clearing in Allama Iqbal Medical Pathology Lab. Vital organs were thinly sliced and they were quickly placed in formalin for 4 hrs and then again for an hour, to allow for natural fixation before

Fig. 1: Sample collection of dissected bird's organs

being processed Histopathologically. It takes 5 hrs to repair tissue. The formalin volume was 20-30 times larger than the size of the tissue. Sections of tissue six micrometers thick were cut with a microtome.

To remove excess wax from the tissues, place a thin layer of albumin on glass slides and attach tissue pieces to them. Next, place the slides in an incubator or slider warmer set to deparaffinize for 15 min at 60-70°C. Slides were mounted with balsam from Canada and covered with coverslips.

Slides were labeled and then placed in storage for microscopic analysis. At the microscope unit, Ghazi University where Nikon's ECLIPSE Ji digital microscope is equipped with a Digital Sight 10 camera used with a high resolution of 6K and switching color and monochrome capture that features a high frame rate for fast focusing on high-definition images.

Ethical consideration

Ethical approval: The study was conducted in compliance with the ethical guidelines and was approved by the Research Ethics Committee, Bahauddin Zakariya University, under approval number Zool 801/23.

Statement of human and animal rights: This research adhered to the ethical standards outlined in the Declaration of Helsinki for studies involving humans and the national guidelines for the care and use of animals.

Statement of informed consent: All participants or their legal representatives provided informed consent before their involvement in the study, as applicable.

Statistical analysis: The degree of covariation in the studied data was found by correlation analysis. The associated data were shown using the Simple Linear Regression (SLR) equation to create dispersed plots. For each parameter, a One-way Analysis of Variance (ANOVA) was applied based on standard error $p>0.5$[33].

RESULTS

Body weights were measured during the summer and winter seasons for both steroids.

Diane-35 (cyproterone acetate-ethinyl estradiol): When comparing the treated birds to the control group, the body weight change displayed a random zigzag pattern. The weight of control group birds increased continuously while the weight of tablet treated birds firstly decreased in 10 day's trial and then increased in 20 day's trial. The intake of food and drink was decreased throughout the first three days of the experiment following the medication administration in the treatment group. The medication had negative effects on the liver. Following medication application, body temperature fluctuated at random. The control group birds' body temperature did not significantly rise. Following medication delivery, hair loss also happened shown in Table 1-4.

IVF-C Inj 5000 IU (human chronic gonadotropin): Birds treated with IVF-C Inj 5000 IU, showed an increase in body weight in 10-day summer and winter trials. Treated birds did not show a reduction in appetite. This drug adversely affected the hair. The hair loss in almost all members of the treated group occurred. Swelling on the gastrointestinal tract was observed. Sign observed on the 4th day of drug administration. At the start, there was a higher mortality rate of birds shown in Table 1-4.

Table 1: Comparison of body weight (g) between steroids treated and untreated *C. coturnix* under 10 day of experimental conditions of summer trial

Parameter	Control	Diane-35	IVF-C Inj	p-value
Initial	134.00±10.700 (120.71-147.29)	97.20±9.783 (85.05-109.35)	97.60±11.632 (83.16-112.04)	0.000*
After 4 days	136.00±11.874 (121.26-150.74)	97.40±4.501 (84.90-109.90)	94.60±12.700 (78.83-110.37)	0.000*
After 7 days	137.60±12.700 (121.83-153.37)	98.40±6.030 (81.66-115.14)	97.20±12.357 (81.86-112.54)	0.000*
After 10 days	139.40±13.520 (122.61-156.19)	99.80±4.200 (88.14-111.46)	98.80±10.826 (85.36-112.24)	0.000*

Data is expressed as Mean±Standard deviation, parentheses show the range of each parameter and p-value indicates statistical results of one-way ANOVA

Table 2: Comparison of body weight (g) between steroids treated and untreated *C. coturnix* under 10 day's experimental conditions of winter trial

Parameter	Control	Diane-35	IVF-C Inj	p-value
Initial	90.80±7.294 (81.74-99.86)	91.60±5.639 (84.60-98.60)	89.00±9.925 (76.68-101.32)	0.867
After 4 days	94.80±7.497 (85.49-104.11)	91.20±4.817 (85.22-97.18)	88.80±9.176 (77.41-100.19)	0.457
After 7 days	99.20±7.014 (90.49-107.91)	90.60±6.107 (83.03-98.18)	93.20±9.985 (80.80-105.60)	0.248
After 10 days	104.00±5.788 (96.81-111.19)	95.00±4.637 (89.24-100.76)	95.20±9.985 (82.80-107.60)	0.119

Data is expressed as Mean±Standard deviation, parentheses show the range of each parameter and p-value indicates statistical results of one-way ANOVA

Table 3: Comparison of body weight (g) between steroids treated and untreated *C. coturnix* under 20 day's experimental conditions of summer trial

Parameter	Control	Diane-35	IVF-C Inj	p-value
Initial	101.00±2.236 (98.22-103.78)	85.60±9.555 (73.74-97.46)	91.00±12.083 (76.00-106.00)	0.053
After 4 days	109.40±1.503 (105.23-113.57)	106.40±14.792 (88.03-124.77)	99.60±17.573 (77.78-121.42)	0.515
After 7 days	115.40±2.074 (112.83-117.97)	117.60±9.450 (105.87-129.33)	122.80±19.537 (98.54-147.06)	0.645
After 10 days	122.00±1.414 (120.24-123.76)	125.40±8.849 (114.41-136.39)	123.20±16.664 (102.51-143.89)	0.884
After 13 days	128.20±3.033 (124.43-131.97)	133.60±8.264 (123.34-143.86)	130.20±16.331 (109.92-150.48)	0.729
After 16 days	129.40±31.30 (125.51-133.29)	129.40±8.649 (118.66-140.14)	130.80±15.770 111.22-150.38)	0.971
After 19 days	130.60±1.288 (127.02-134.18)	131.80±7.294 (122.74-140.86)	129.60±15.630 (110.19-149.01)	0.942

Data is expressed as Mean±Standard deviation, parentheses show the range of each parameter and p-value indicates statistical results of one-way ANOVA

Table 4: Comparison of body weight (g) between steroids treated and untreated *C. coturnix* under 20 day's experimental conditions of winter trial

Parameter	Control	Diane-35	IVF-C Inj	p-value
Initial	133.60±3.209 (129.62-137.58)	180.40±7.232 (171.42-189.38)	181.00±11.467 (166.76-195.24)	0.000*
After 4 days	140.60±1.673 (138.52-142.68)	177.00±16.926 (155.98-198.02)	187.80±11.904 (173.02-202.58)	0.000*
After 7 days	148.20±3.114 (144.33-152.07)	182.60±18.133 (160.09-205.11)	193.00±12.903 (176.98-209.02)	0.000*
After 10 days	145.80±21.592 (118.99-172.61)	187.60±17.184 (166.26-208.94)	197.80±12.153 (182.71-212.89)	0.001*
After 13 days	164.40±5.683 (157.34-171.46)	193.40±17.501 (171.67-215.13)	204.60±12.178 (189.48-219.72)	0.001*
After 16 days	163.60±6.181 (156.13-171.47)	189.80±16.769 (168.98-210.62)	196.80±3.768 (192.12-201.48)	0.001*
After 19 days	164.80±6.181 (157.13-172.47)	187.20±15.991 (167.35-207.05)	187.40±15.437 (168.23-206.57)	0.030*

Data is expressed as Mean±Standard deviation, parentheses show the range of each parameter and p-value indicates statistical results of one-way ANOVA

Mortality rate: Steroids posed adverse effects on the physical conditions of birds. Mortality is frequently observed in summer birds treated with cyproterone acetate. Birds died on the 5th, 7th and 10th day treated with Diane-35 in the summer season. One bird died on the 8th day treated with IVF-C Inj 5000 IU shown in Table 1-4.

The data shows a significant reduction in the measured parameter in both Diane-35 and IVF-C Injection groups compared to the control group at all time points (4, 7 and 10 days), with p-values less than 0.000, indicating statistical significance. The control group consistently showed higher values at each time point compared to the other two treatments shown in Table 1.

Table 2 shows no significant differences across the three groups (Control, Diane-35 and IVF-C) at various time points (initial, after 4, 7 and 10 days), as indicated by the p-values above 0.05. At all time points, the measurements for each group are similar, suggesting no significant impact of the interventions compared to the control.

Table 3 shows that the three groups (Control, Diane-35 and IVF-C) exhibited similar values across various time points, with no significant differences between them ($p>0.05$ for all time points). The measurements gradually increased over time, but the differences between groups remained statistically insignificant throughout the 19-day period.

Table 4 shows significant increases in the measured parameter over time across all groups. Both Diane-35 and IVF-C Inj groups demonstrated higher values compared to the control group at every time point (from initial to 19 days), with statistically significant differences ($p<0.05$) observed at all intervals except for the 19-day mark, where the difference between IVF-C Inj and the control group was smaller but still significant ($p = 0.030$). The most notable increases were observed after 13 and 16 days.

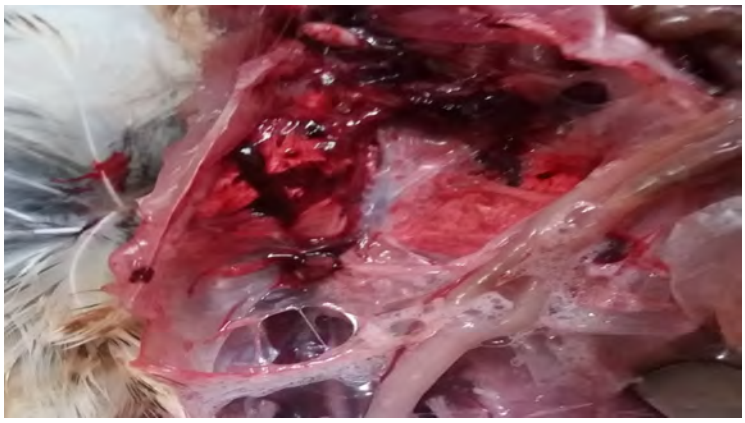

Fig. 2: Bird treated with tablet showed scum web on GIT

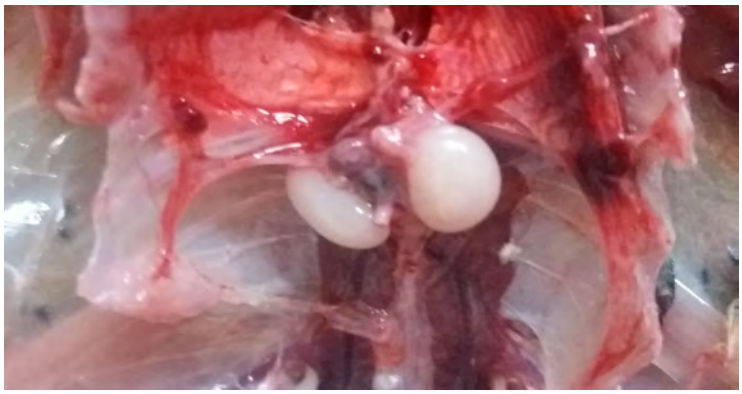

Fig. 3: Bird treated with Diane-35 showed enlarged testis

Patho-morphological observations: Except for one, every bird treated with Diane-35 exhibited a notable difference in their hearts and muscles when compared to the control group. But the testicles and liver displayed some symptoms. One member's muscles were excessively red in the event of a pill on the eighteenth day of slaughter during the winter testing. Upon the sixteenth day of slaughter, scum on the GIT was shown in Fig. 2.

All individuals in the treated groups had normal testicles after ten days of taking the pill. One bird displayed more testicular growth after twenty days shown in Fig. 3.

When compared to the control group, the hearts of all birds treated with Diane-35 showed no appreciable alterations. The summer trial-treated birds showed normal muscle tone and no signs of edema. In the summer trial-treated birds, the liver color shifted from red to yellow, causing damage to the left lobe of the liver; the same symptoms were not seen in the winter trial-treated animals. This medication negatively affected the liver. The liver of the twenty-day IVF-C 5000 IU Inj administered group was larger and had a softer consistency than the liver of the control group. On the fourteenth day after injection, there was evidence of gastrointestinal edema during slaughter. Every member of the cohort had normal cardiac and muscle function shown in Fig. 4 and 5.

When it came to both steroids, the kidneys were normal. Among the birds in the treatment group, one had black testicles and the others were normal shown in Fig. 6.

One bird had liver that was yellow-greenish rather than red in the group besides heart enlargement that received IVF-C Injection 5000 IU treatment shown in Fig. 7 and 8.

Histopathological findings

Heart muscles: A modest inflammatory infiltration was observed upon microscopic analysis of the treated group's heart and muscle-dyed slides. In control groups, no significant histological alterations were seen. Smooth muscle fibers were visible in the control heart's histology. Longer muscle fibers were visible in the control muscles. The core nucleus of muscle fibers was rounded shown in Fig. 9.

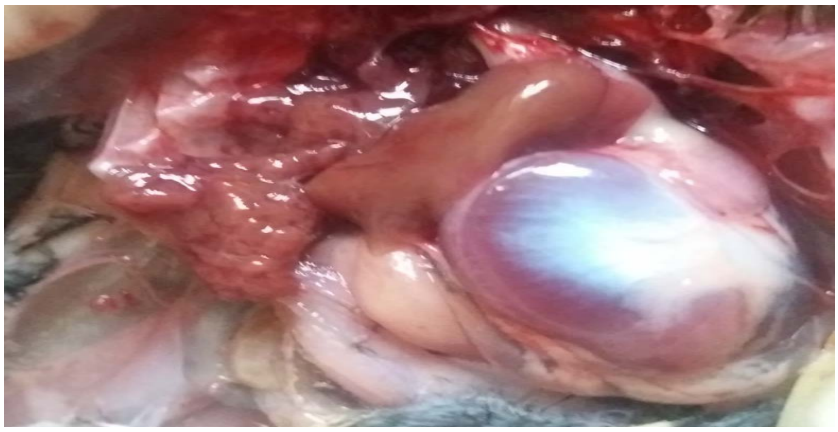

Fig. 4: Bird treated with Diane-35 showed liver damage

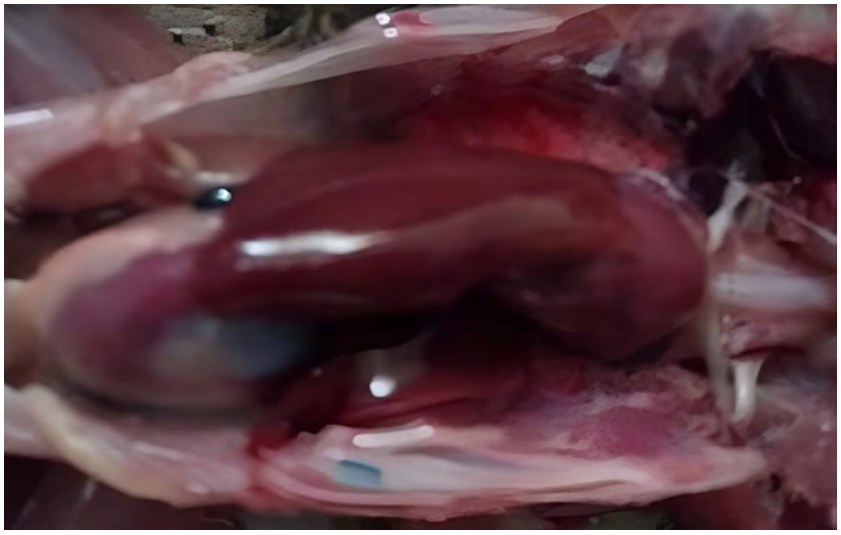

Fig. 5: Bird treated with IVF-C Injection had liver enlargement

Fig. 6: Bird treated with IVF-C Injection had blackening of testis

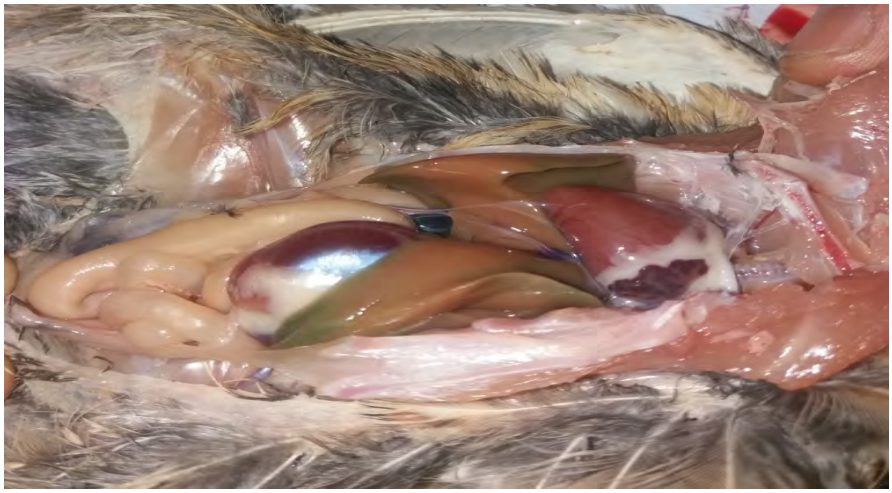

Fig. 7: Bird treated with IVF-C Injection had yellow greenish liver

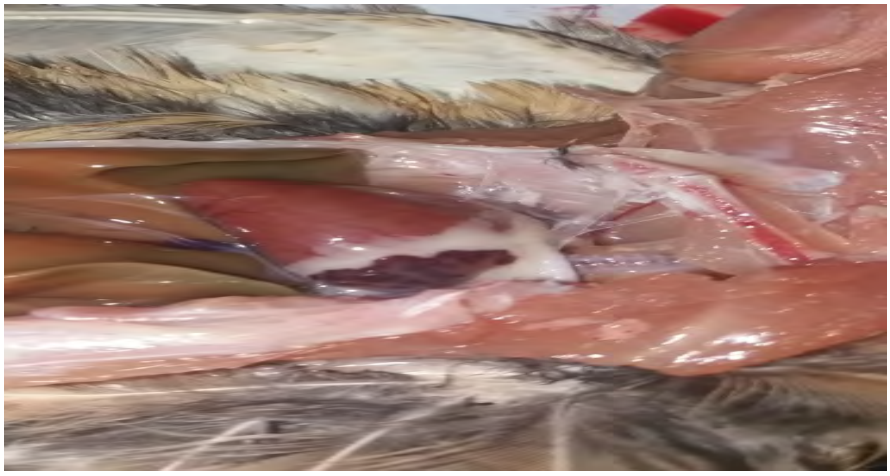

Fig. 8: Bird treated with IVF-C Injection showing heart enlargement

Fig. 9: Control group heart showing elongated muscle fibers (40X)

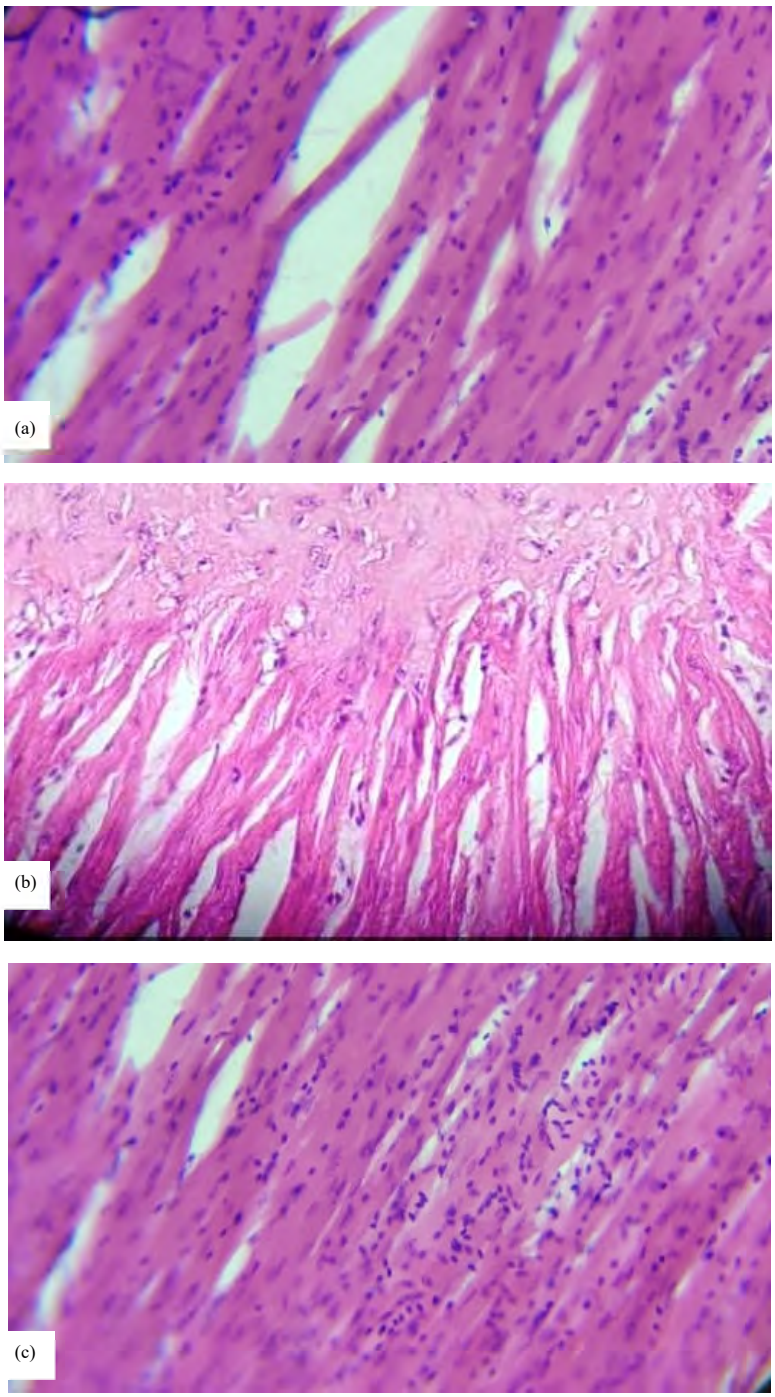

Fig. 10(a-c): Cross-sectional view of control group heart (40X), (a) Mild inflammation, (b) Ectatic blood vessels and (c) Scattered lymphocytes

Few thin-walled blood vessels of the control group were interspersed and no inflammatory infiltration was noted. Few muscle fibers of control group members were intact; otherwise, other members had normal muscle fibers when observed histologically shown in Fig. 10a-c.

In the Diane-35 treated group, heart muscles fibers showed slightly changes from control group heart muscles fibers. Muscles cells were normal with central nuclei. Some blood vessel slightly dilated and showed inflammatory infiltrate shown in Fig. 11a-c.

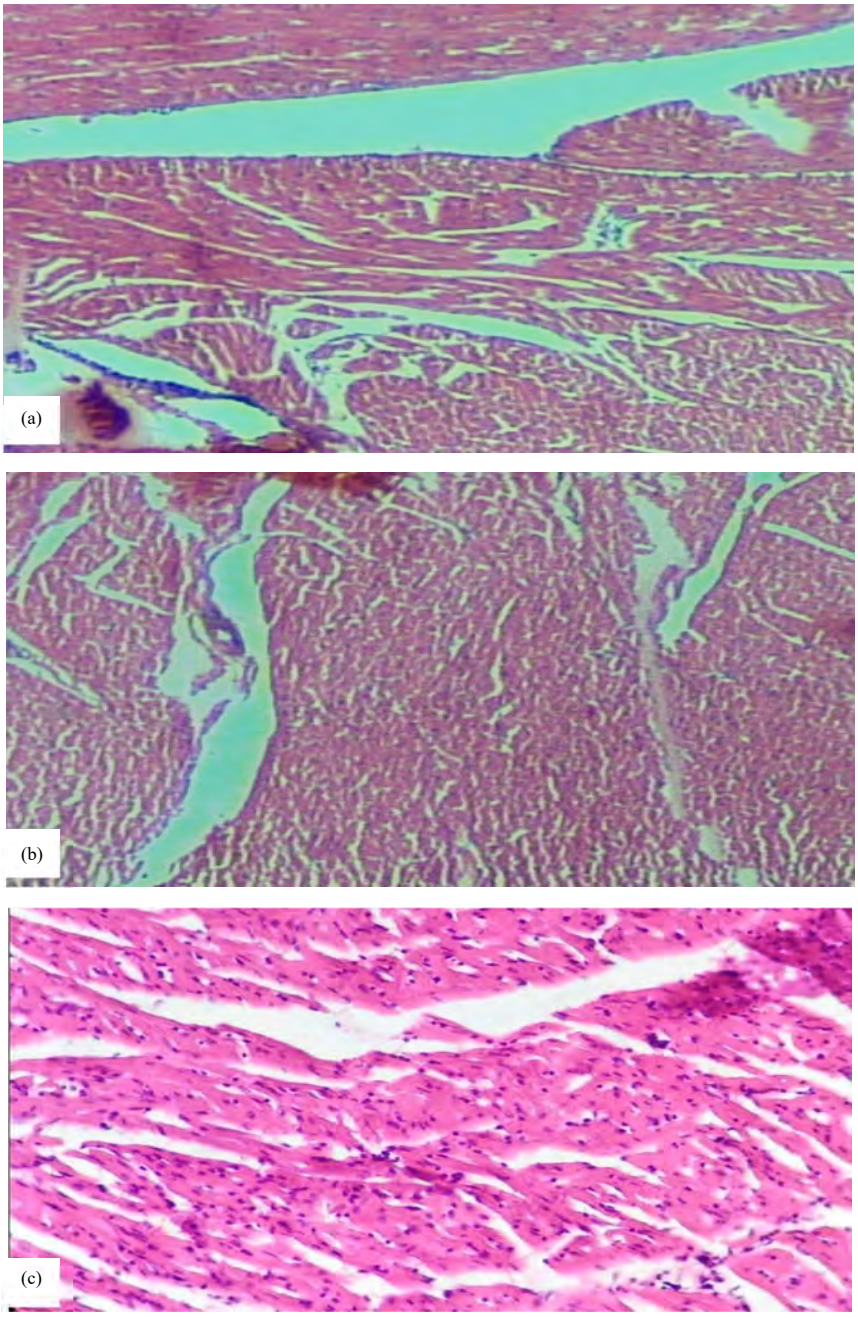

Fig. 11(a-c): Cross-sectional view of Diane-35 treated group heart (40X), (a) Dilated vessel, (b) Inflammation and (c) Lymphatic infiltrate

In IVF-C Inj 5000 IU treated groups, heart muscles showed mild congestion and mild scattered lymphocytic infiltrate shown in Fig. 12a-c.

Body muscles: All control groups' muscles displayed centrally located muscle fiber elongation. Longer muscular fibers were visible upon microscopic inspection of the injected muscles. The central nuclei of these muscle fibers were rounded and normal. Only one or two lymphocyte foci were seen. No particular muscular anomalies were found. There were not many inflammatory cells found shown in Fig. 13.

In IVF-C Inj 5000 IU treated groups, body muscles showed inflammation and foci of lymphocytes shown in Fig. 14a-b.

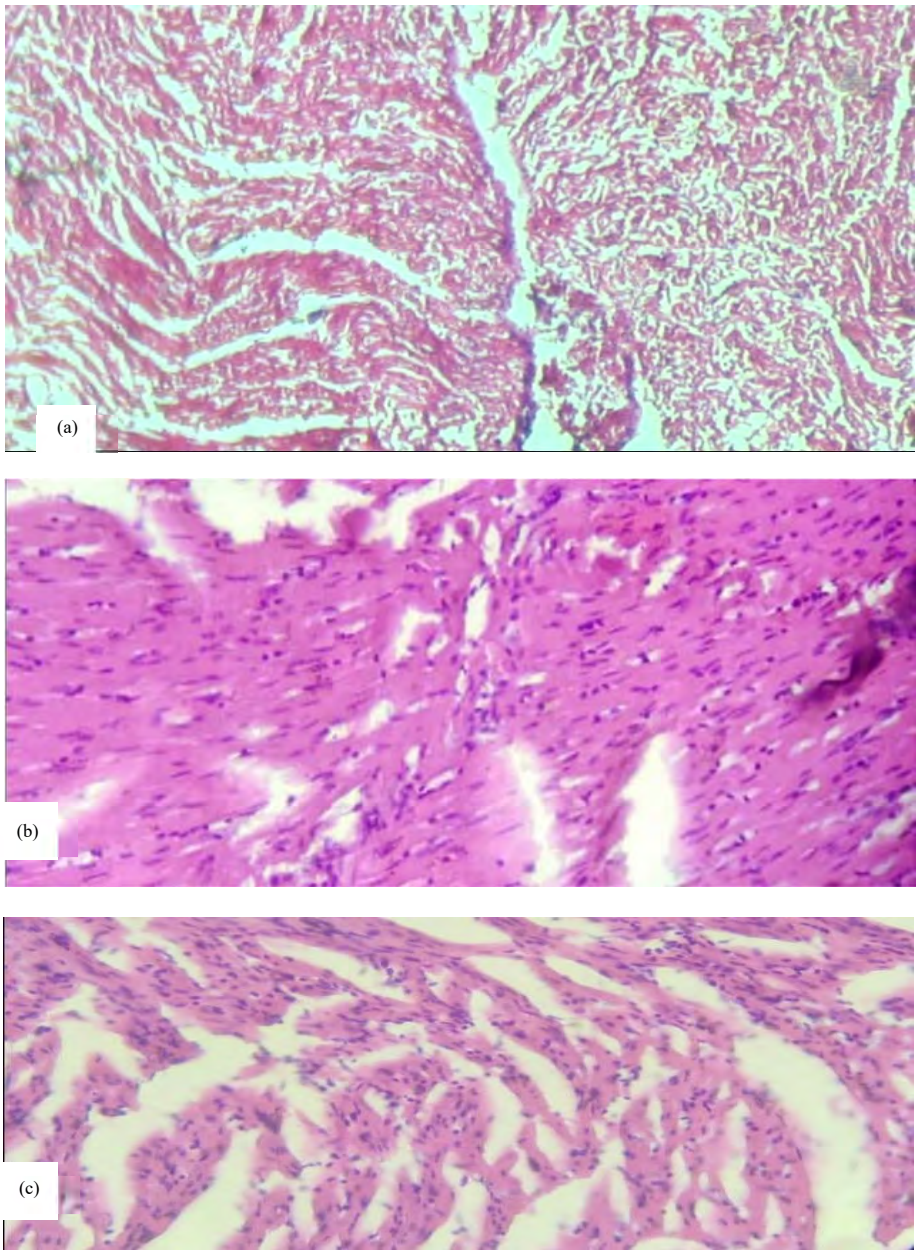

Fig. 12(a-c): Cross-sectional view of heart of IVF-C Inj treated group (40X), (a) Inflammatory infiltrate, (b) Lymphocytes and (c) Mild swelling

The group that received Diane-35 treatment exhibited 40X inflammatory infiltration in their muscles, which included lymphocytes and inflammation shown in Fig. 15a-b.

Statistical findings: The correlated Diane-35 vs IVF-C data was higher near to the whole integer right number than correlated control vs both drugs. Correlated each drug vs control data was near the same. All Simple Linear Regression (SLR) curves expressed high positive regressed except Diane-35 vs IVF-C scattered plot data that was very high shown in Table 5 and Fig. 16.

DISCUSSION

Steroids are widely employed in both human and veterinary medicine for various objectives, including muscle augmentation[34], development promotion and muscle strength enhancement. These drugs are classified into two

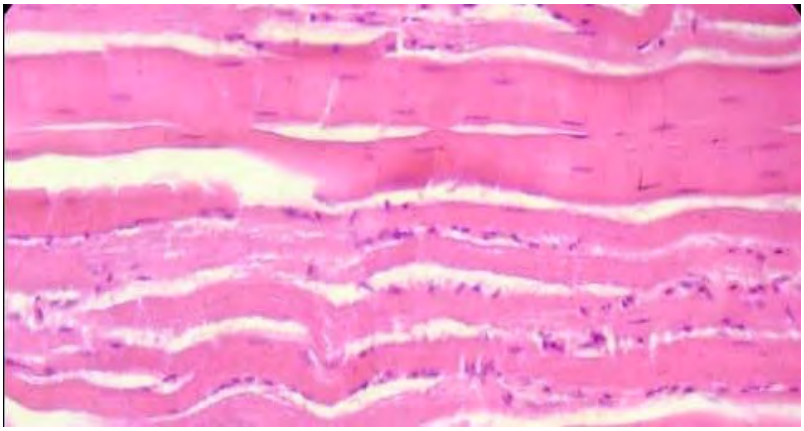

Fig. 13: Muscles of control group showing normal muscles fiber (40X)

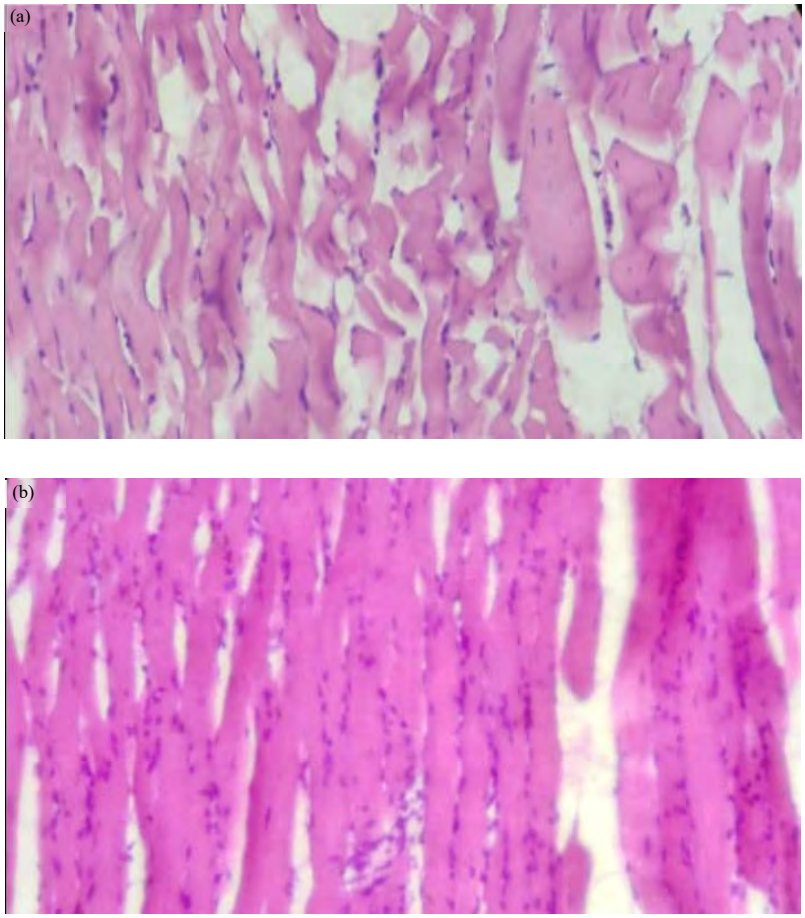

Fig. 14(a-b): IVF-C Injection treated group muscles showing foci of lymphocytes (40X), (a) Lymphocytes and (b) Inflammation

Table 5: Correlation among control and both drugs' data

Parameter	Control	Diane-35	IVF-C
Control	-	0.792	0.784
Diane-35	0.792	-	0.996
IVF-C	0.784	0.996	-

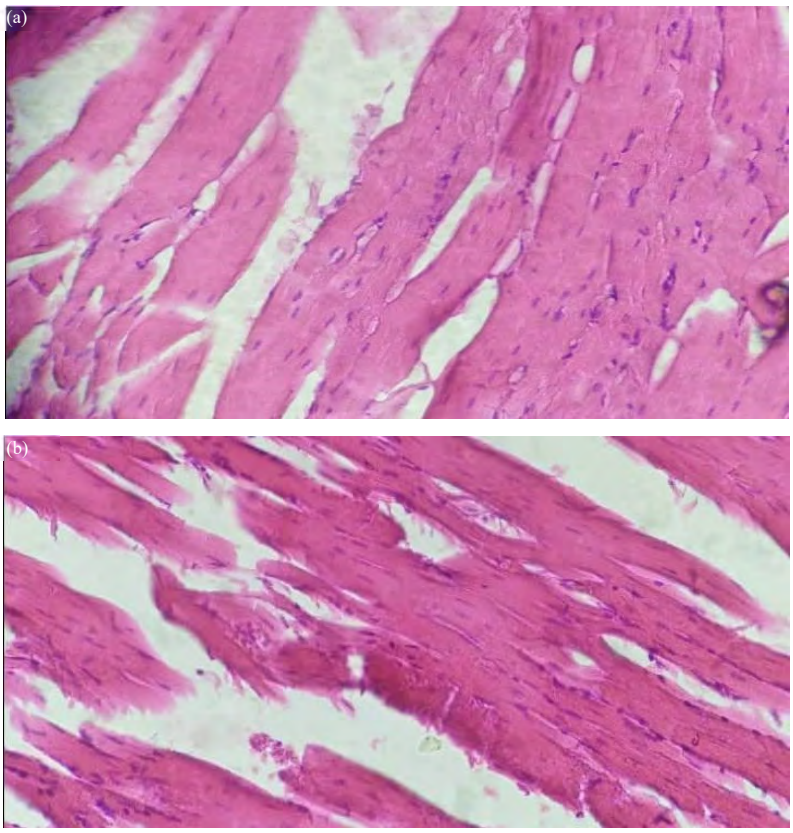

Fig. 15(a-b): Muscles of Diane-35 treated group showing inflammatory infiltrate (40X), (a) Lymphocytes and (b) Inflammation

primary categories: Anabolic steroids and corticosteroids. While they possess therapeutic benefits when utilized correctly, their misuse or overuse can result in toxic consequences. Due to high correlated results between two drugs, they suggested to have the most compatible effects with the deepest action. Comprehending the potential risks associated with specific steroids is essential for safeguarding both human and animal well-being.

Both fungi and viruses can cause infection. Research on inflammation in birds has advanced in a number of areas. Compared to mammals, birds typically experience inflammatory processes significantly more quickly, which can result in persistent lesions[35]. To further categorize the avian inflammatory system and predict when visibilities may act pharmacologically in these systems, however, extensive research is required. Mammals and birds have similar inflammatory responses, yet they also differ from one another[36].

After receiving therapeutic doses of specific steroids, such as Diane-35 containing cyproterone acetate and IVF-C Inj 5000 IU containing human chronic gonadotropin, the post-treatment clinical symptoms seen in these birds were loss of appetite, hair loss, intestinal filth, and heart enlargement as shown in Fig. 2 and 8. In order to mitigate the seasonal bias, the experimental trials were conducted twice, in the summer and winter. As the members of the control group were normal under the same environmental conditions, it may be explained by the sporadic effects of a significant average increase in body temperature in conjunction with steroid therapy. All clinical symptoms were similar in both studies, with the exception of hair loss in the summer study. A study that used a therapeutic dose rate of 0.1 mg/kg of cyproterone acetate demonstrated mortality on the 5th day after treatment, which is consistent with the rare publications that describe clinical indications of steroid toxicity in avian species. The fact that the experiment's steroids did not prove to be fatal while short-term and low-dose steroid use are safest. Several negative effects arise from long-term high dose consumption[37].

The histology and clinical characteristics in all of the current trials, such as body weight, body temperature, testis size, hair loss, liver and kidneys, showed notable differences when compared to the control group[38]. Prolonged cyproterone acetate therapy has negative side effects on the

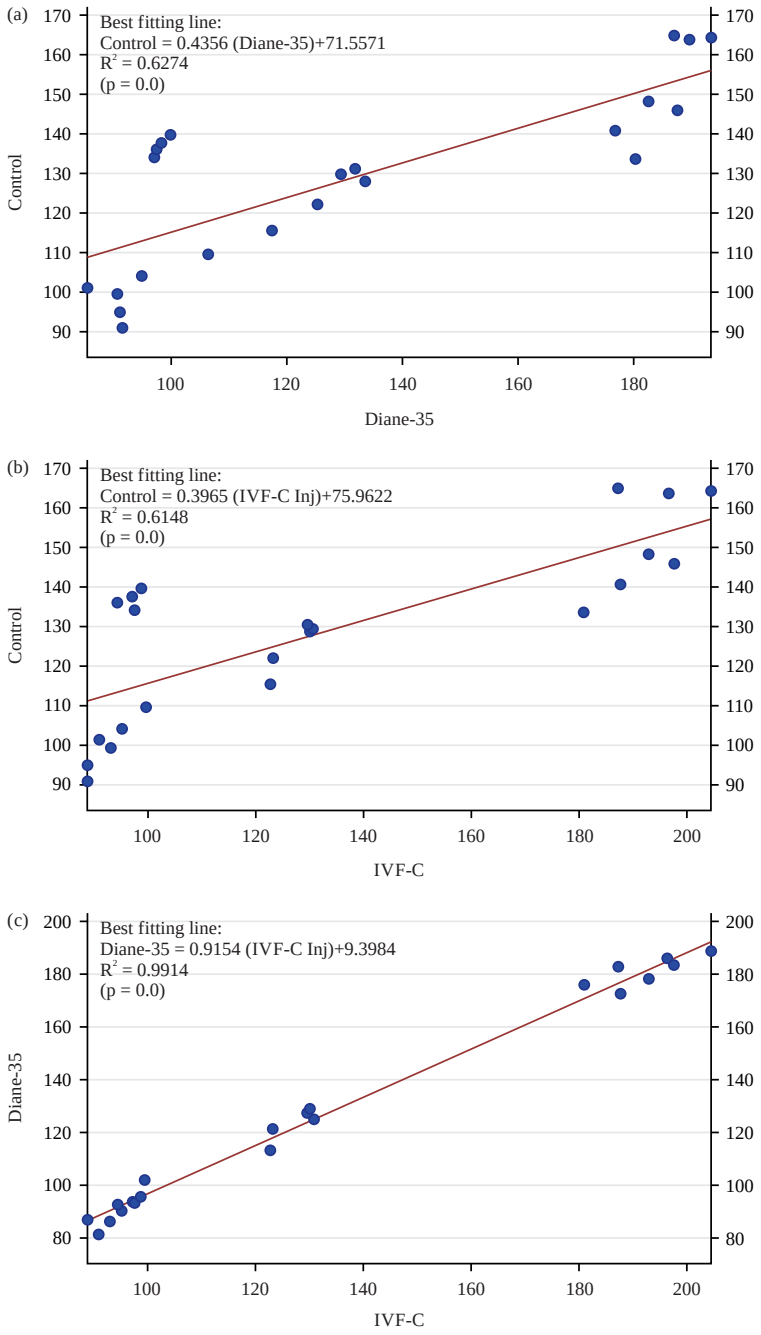

Fig. 16(a-c): Simple linear regression (SLR) curves among control and both drugs, (a) Control vs Diane-35, (b) Control vs IVF-C and (c) Diane-35 vs IVF-C

liver that can lead to hepatocellular cancer[39]. It is advised that patients receiving long-term cyproterone acetate medication have their livers checked if they show any indications of proliferative liver alteration[40].

In vitro and *in vivo* studies have demonstrated that cyproterone acetate, an active ingredient in some contraceptive and anti-androgenic drugs, induces DNA repair synthesis in rat hepatocytes. Oral dosages of 0.1 and 30 mg/kg were given to female rats, whereas doses of 1 to 100 mg/kg were given to male rats. Production of DNA adducts during the long-term synthetic steroid therapy of rats involved in the development of hepatic tumors[41].

Conversely, the effect of cyproterone acetate in treated common quail was observed in both summer and winter trials.

In the summer trial when a therapeutic dose of cyproterone acetate was given to a bird for about 20 days, the left lobe of the liver was damaged as shown in Fig. 4. In the winter trial liver was slightly enlarged in some members of the group while others remained normal. In the case of IVF-C Injection 5000 IU extra enlargement of liver[42] and change in color of the liver as shown in Fig. 5 and 7, respectively, observed in the summer trial during necropsy. In winter trials birds treated with human chronic gonadotropin had a soft consistency of liver as compared to untreated birds.

Male Japanese quail were affected behaviorally and morphologically by cyproterone acetate; all birds at all dosages displayed a decrease in cloacal gland size and copulation[43]. In both the summer and winter trials of our investigation, there is no decline in the size of the cloacal gland during the experiment. In the case of both steroids, treated birds displayed normal cloacal size at therapeutic levels. Variation in result was due to the response of birds to the efficiency of various steroids.

The current investigation revealed notable variations in hair development. In a bird treated with human chronic gonadotropin and cyproterone acetate for 20 days, hair loss was seen. In summer studies, hair loss increased dramatically, whereas in winter trials, it dropped significantly. There was reduced hair loss in the groups treated with cyproterone acetate as compared to human chronic gonadotropin. Women with hirsute skin who had cyproterone acetate treatment saw a quantitative change in body hair development. Following two therapy cycles, there was a noticeable reduction in the average hair diameter and the rate of hair growth on the thigh area.

There was a noticeable variation in the severity of clinical disorders and mortality rates at different therapeutic dose levels. In the winter trial, the mortality rate was lower in the groups treated with cyproterone acetate and was at its highest in the IVF-C Injection 5000 IU treated group. However, in the summer, the mortality rate was higher in the birds treated with Diane-35. Variations in clinical symptom severity and mortality indicating a particular immune response may be caused by differences in the medicines administered to the subjects.

In the present study, mortality was examined at the 6th, 8th, 11th and 14th day of treatment in 20 days winter trial birds treated with human chronic gonadotropin and on 8th day in ten days summer trial birds treated with the same drug. In 1990, another study documented alterations in albino rats' testicular tissue, Sertoli cells, Leydig cells, germ cells and spermatid after administering a single dosage of cyproterone acetate (1 mg/100 g) for 60 days[44]. In every trial, the treated birds' testicles were larger than usual, yet in certain cases, they were smaller. In the winter trials, the testis of the bird treated with cyproterone acetate had a larger size than usual and the testis of the human chronic gonadotropin group turned black in comparison to the control group.

The bird may be overheating due to bleeding from its nose after treatment. The bird died as a consequence of its body temperature rising over average.

Both the microtomy results and the physical examination showed that the heart and muscles of all treatment groups did not differ significantly from the control group. This suggested that the toxicity of steroids did not affect the heart or muscles. The cardiac muscles of the treated group of birds displayed dilated arteries at the periphery along with dispersed lymphocytes in the histopathological investigation. The treated group's muscle fibers in the current study had mild inflammation, rounder central nuclei and elongation.

The current studies showed some evidence of liver damage as shown in Fig. 4, about the extremely rare but real occurrence of nephrotoxic side effects from cyproterone acetate, including the formation of liver tumors in rats, with a greater prevalence in females. The observed effect was specifically related to a tumor-promoting mechanism peculiar to rodents, as evidenced by the high hepato-mitogenic activity of cyproterone acetate. More recent research revealed that cyproterone acetate is sex-specifically triggered to a DNA-damaging intermediate in the liver of female rats, causing increased levels of micronuclei and gene mutations, DNA adduct formation and DNA repair induction.

In a liver foci experiment, cyproterone acetate showed tumor-initiating capability in female rats but not in male rats, which is consistent with a sex-specific genotoxicity[45]. The same involutive processes typify the de-differentiation of renal tubules under these circumstances as those brought on by castration, with the exception that the effects of cyproterone acetate start to show after 14 days. In contrast, the first indications of involution become apparent after 7 days following castration. The extreme structural differences impact the Golgi apparatus, the rough endoplasmic reticulum and the nucleoli. The outcomes demonstrated that giving male sticklebacks cyproterone acetate had an inhibitory effect on renal target cells[46]. Seemingly indistinguishable from the alterations brought on by a deficiency in male sex hormones, this medication could be a good alternative to castrating fish.

Gross observations revealed that the steroid-treated therapeutic dose groups had edematous brain tissue, pale liver and kidneys that were swollen and mottled. Birds given medication after treatment exhibited significant pathological abnormalities, including congestion, diffuse focal necrosis in

the liver and gradual granular degenerative changes. The kidneys showed signs of severe tubular nephrotic alterations, increased glomerular cellularity and glomeruli adhering to Bowman's capsule. In the current investigation, similar minor alterations in liver cells and color changes were investigated. When compared to the control group of birds, the liver of the treated group of birds had significant changes under a microscope. Hepatocytes displayed dilated vascular spaces and cytoplasmic vacuolation cytoplasmic hepatocytes had a reduction in eosinophils. The treated group's kidney cells displayed moderate edema and dilatation.

Other previous studies including different steroid treatments against quail illustrated the same results; there was no appetite for food intake for quails treated with exogenous corticosterone. There was no body weight increment for the treated groups. On the other hand, dexamethasone-treated quail groups expressed low fertility and less egg weight while there was an increment in testis organs with swollen areas with non-effective sperm. Furthermore, estradiol benzoate and testosterone propionate caused a complete regression of quail testis while dihydrotestosterone didn't affect testicular or body weights[47,48].

CONCLUSION

The study on the effects of IVF-C Inj 5000 IU on common quails revealed that steroids significantly impacted all treated groups, causing symptoms such as hair loss, appetite loss, depression and body temperature fluctuations. Mortality occurred in the human chronic gonadotropin and cyproterone acetate-treated groups, with injections causing higher mortality in winter and tablets in summer. While there were no significant changes in skeletal muscles or hearts, cyproterone acetate treatment led to liver damage and testicular enlargement. Overall, the study highlighted both the therapeutic and pathological effects of steroid treatments.

SIGNIFICANCE STATEMENT

This study gave the spot on the use of steroids as cures for bird treatments. It focused on two steroids; Diane-35 and IVF-C Inj 5000 IU on common quails. This study realized the harmful effects of these steroids by applying them at different seasons within interval times. These durations allow the execution of experiments to be optimum. It didn't encourage excessive use of steroids due to the negative impacts on histological parenchyma of organs. A sufficient studied dose should be applied to obtain the desired results without any side effects. Treatment of any artificial medicine should be taken carefully. Follow-up with other seasons or different birds will permit the science to take a fateful decision on the degree of steroid treatment.

REFERENCES

1. Latino, D., M. Venditti, S. Falvo, G. Grillo and A. Santillo *et al*., 2024. Steroidogenesis upregulation through mitochondria-associated endoplasmic reticulum membranes and mitochondrial dynamics in rat testes: The role of D-aspartate. Cells, Vol. 13. 10.3390/cells13060523.
2. Vazakidou, P., S. Evangelista, T. Li, L.L. Lecante and K. Rosenberg *et al*., 2024. The profile of steroid hormones in human fetal and adult ovaries. Reprod. Biol. Endocrinol., Vol. 22. 10.1186/s12958-024-01233-7.
3. Kim, C., E. Jeong, Y.B. Lee and D. Kim, 2024. Steroidogenic cytochrome P450 enzymes as drug target. Toxicol. Res., 40: 325-333.
4. Bremer, A.A. and W.L. Miller, 2014. Regulation of Steroidogenesis. In: Cellular Endocrinology in Health and Disease, Ulloa-Aguirre, A. and P.M. Conn (Eds.), Academic Press, Cambridge, Massachusetts, ISBN: 9780124081345, pp: 207-277.
5. Diotel, N., T.D. Charlier, C.L. d'Hellencourt, D. Couret and V.L. Trudeau *et al*., 2018. Steroid transport, local synthesis, and signaling within the brain: Roles in neurogenesis, neuroprotection, and sexual behaviors. Front. Neurosci., Vol. 12. 10.3389/fnins.2018.00084.
6. Baer, J., R. Lansford and K. Cheng, 2015. Japanese Quail as a Laboratory Animal Model. In: Laboratory Animal Medicine, Fox, J.G., L.C. Anderson, G.M. Otto, K.R. Pritchett-Corning and M.T. Whary (Eds.), Academic Press, Cambridge, Massachusetts, ISBN: 9780124095274, pp: 1087-1108.
7. Agina, O.A., W.S. Ezema and E.M. Iwuoha, 2017. The haematology and serum biochemistry profile of adult Japanese quail (*Coturnix coturnix japonica*). Notulae Sci. Biol., 9: 67-72.
8. Cheng, K.M., D.C. Bennett and A.D. Mills, 2010. The Japanese Quail. In: The UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, Hubrecht, R. and J. Kirkwood (Eds.), The Universities Federation for Animal Welfare Wheathampstead, Hertfordshire, England, ISBN: 9781444318777, pp: 655-673.
9. Dubrovsky, B.O., 2005. Steroids, neuroactive steroids and neurosteroids in psychopathology. Prog. Neuro-Psychopharmacol. Biol. Psychiatry, 29: 169-192.
10. Heimovics, S.A., B.C. Trainor and K.K. Soma, 2015. Rapid effects of estradiol on aggression in birds and mice: The fast and the furious. Integr. Comp. Biol., 55: 281-293.
11. Robson, K., 2024. Pharmacokinetic parameters: Understanding the dynamics of drug absorption, distribution, metabolism and excretion. J. Formulation Sci. Bioavailability, Vol. 8. 10.37421/2577-0543.2024.8.194.

12. Benedetti, M.S., R. Whomsley, I. Poggesi, W. Cawello and F.X. Mathy *et al*., 2009. Drug metabolism and pharmacokinetics. Drug Metab. Rev., 41: 344-390.
13. Vinarov, Z., M. Abdallah, J.A.G. Agundez, K. Allegaert and A.W. Basit *et al*., 2021. Impact of gastrointestinal tract variability on oral drug absorption and pharmacokinetics: An UNGAP review. Eur. J. Pharm. Sci., Vol. 162. 10.1016/j.ejps.2021.105812.
14. Schlinger, B.A., 2015. Steroids in the avian brain: Heterogeneity across space and time. J. Ornithol., 156: 419-424.
15. Saldanha, C.J. and B.A. Schlinger, 2008. Steroidogenesis and Neuroplasticity in the Songbird Brain. In: Neuroactive Steroids in Brain Function, Behavior and Neuropsychiatric Disorders: Novel Strategies for Research and Treatment, Ritsner, M.S. and A. Weizman (Eds.), Springer, Dordrecht, Netherlands, ISBN: 978-1-4020-6854-6, pp: 201-216.
16. Tsutsui, K., 2011. Neurosteroid biosynthesis and function in the brain of domestic birds. Front. Endocrinol., Vol. 2. 10.3389/fendo.2011.00037.
17. Brenowitz, E.A., 2015. Transsynaptic trophic effects of steroid hormones in an avian model of adult brain plasticity. Front. Neuroendocrinol., 37: 119-128.
18. Wang, L., X. Luo, Q. Wang, Q. Lv, P. Wu, W. Liu and X. Chen, 2021. Fertility-preserving treatment outcome in endometrial cancer or atypical hyperplasia patients with polycystic ovary syndrome. J. Gynecol. Oncol., Vol. 32. 10.3802/jgo.2021.32.e70.
19. Uras, R., M. Orrù, F. Pani, M.F. Marotto and M. Pilloni *et al*., 2010. Endocrinological, metabolic and clinical features of treatment with oral contraceptive formulation containing ethinylestradiol plus chlormadinone acetate in nonobese women with polycystic ovary syndrome. Contraception, 82: 131-138.
20. Ruan, X., J. Song, M. Gu, L. Wang, H. Wang and A.O. Mueck, 2018. Effect of Diane-35, alone or in combination with orlistat or metformin in Chinese polycystic ovary syndrome patients. Arch. Gynecology Obstet., 297: 1557-1563.
21. de Souza, T.O., Â.J. Ben, J.M. van Dongen, J.E. Bosmans and J.S.L. da Cunha-Filho, 2023. Effectiveness and cost-effectiveness of minimal ovarian stimulation *in-vitro* fertilization versus conventional ovarian stimulation in poor responders: Economic evaluation alongside a propensity score adjusted prospective observational study. JBRA Assisted Reprod., 27: 204-214.
22. Kyle, U.G., L.S. Shekerdemian and J.A. Coss-Bu, 2015. Growth failure and nutrition considerations in chronic childhood wasting diseases. Nutr. Clin. Pract., 30: 227-238.
23. Cai, Y., Z. Song, X. Wang, H. Jiao and H. Lin, 2011. Dexamethasone-induced hepatic lipogenesis is insulin dependent in chickens (*Gallus gallus domesticus*). Stress, 14: 273-281.
24. Adkins-Regan, E., 2020. Sexual and pairing partner preference in birds and other animals. Horm. Behav., Vol. 118. 10.1016/j.yhbeh.2019.104646.
25. Sur, S., A. Sharma, S.K. Bhardwaj and V. Kumar, 2020. Involvement of steroid and antioxidant pathways in spleen-mediated immunity in migratory birds. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., Vol. 250. 10.1016/j.cbpa.2020.110790.
26. Paitz, R.T. and M.B. Dugas, 2022. Steroid levels in frog eggs: Manipulations, developmental changes, and implications for maternal steroid effects. J. Exp. Zool. Part A: Ecol. Integr. Physiol., 337: 293-302.
27. Kostellow, A.B. and G.A. Morrill, 2008. Progesterone and subsequent polar metabolites are essential for completion of the first meiotic division in amphibian oocytes. Mol. Cell. Endocrinol., 291: 50-56.
28. Hrabia, A., 2022. Reproduction in the Female. In: Sturkie's Avian Physiology, Scanes, C.G. and S. Dridi (Eds.), Academic Press, United States, ISBN: 978-0-12-819770-7, pp: 941-986.
29. Javůrková, V.G. and I. Mikšík, 2023. New insights into the relationships between egg maternal components: The interplays between albumen steroid hormones, proteins and eggshell protoporphyrin. Comp. Biochem. Physiol. Part A: Mol. Integr. Physiol., Vol. 279. 10.1016/j.cbpa.2023.111401.
30. Zain Ul-Abideen, H.I. Ahmad, M. Nadeem, A.A. Khan and M. Imran *et al*., 2022. The therapeutic effect of bromocriptine as mesylate and estradiol valerate on serum and blood biochemistry of common quails. Poult. Sci., Vol. 101. 10.1016/j.psj.2021.101552.
31. Ritchie, B.W., J.G. Harrison and R.L. Harrison, 1994. Avian Medicine: Principles and Application. 2nd Edn., Wingers Pub., Lake Worth Beach, Florida, ISBN: 9780963699602, Pages: 1384.
32. Möstl, E., S. Rettenbacher and R. Palme, 2005. Measurement of corticosterone metabolites in birds' droppings: An analytical approach. Ann. N. Y. Acad. Sci., 1046: 17-34.
33. Miller, A., J. Panneerselvam and L. Liu, 2022. A review of regression and classification techniques for analysis of common and rare variants and gene-environmental factors. Neurocomputing, 489: 466-485.
34. Stolker, A.A.M. and U.A.T. Brinkman, 2005. Analytical strategies for residue analysis of veterinary drugs and growth-promoting agents in food-producing animals-A review. J. Chromatogr. A, 1067: 15-53.
35. Evans, N.A., 2004. Current concepts in anabolic-androgenic steroids. Am. J. Sports Med., 32: 534-542.
36. Koutsianos, D., L. Athanasiou, D. Mossialos and K.C. Koutoulis, 2020. Colibacillosis in poultry: A disease overview and the new perspectives for its control and prevention. J. Hellenic Vet. Med. Soc., 71: 2425-2436.
37. Kaiser, P., L. Rothwell, S. Avery and S. Balu, 2004. Evolution of the interleukins. Dev. Comp. Immunol., 28: 375-394.

38. de Vleeschhauwer, F., K. Casteels, I. Hoffman, M. Proesmans and A. Rochtus, 2024. Systemic adverse events associated with locally administered corticosteroids. Children, Vol. 11. 10.3390/children11080951.
39. Qi, J., Y.Q. Gao, S.J. Kang, C. Liu and J.M. Gao, 2023. Secondary metabolites of bird's nest fungi: Chemical structures and biological activities. J. Agric. Food Chem., 71: 6513-6524.
40. Chitturi, S. and G.C. Farrell, 2013. Adverse Effects of Hormones and Hormone Antagonists on the Liver. In: Drug-Induced Liver Disease, Kaplowitz, N. and L.D. DeLeve (Eds.), Academic Press, Cambridge, Massachusetts, ISBN: 9780123878175, pp: 605-619.
41. Muir, C.A., M. Guttman-Jones and E.J. Man, 2024. Effects of gender affirming hormone treatment in transgender individuals-A retrospective cohort study. Endocrine, 85: 370-379.
42. Martelli, A., F. Mattioli, M. Ghia, E. Mereto and G. Brambilla, 1996. Comparative study of DNA repair induced by cyproterone acetate, chlormadinone acetate and megestrol acetate in primary cultures of human and rat hepatocytes. Carcinogenesis, 17: 1153-1156.
43. Ali, S., M. Zubair, S. Umar, M. Akhtar and Z. Iqbal *et al*., 2017. Effects of clomiphene citrate (CC) and Human Chorionic Gonadotropin (hCG) on hormonal profile, serum biochemical constituents, and oxidative stress in pre-pubertal Sahiwal heifers. Indian J. Anim. Res., 52: 959-963.
44. Zhang, X., J. Li, S. Chen, N. Yang and J. Zheng, 2023. Overview of avian sex reversal. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms24098284.
45. Mäusle, E., F. Stadtler and D. Stenger, 1982. Effects of cyproterone acetate and oestradiol benzoate on the rat testis: Morphometric study after treatment over 35 days. Pathol. Res. Pract., 173: 218-224.
46. Deml, E., L.R. Schwarz and D. Oesterle, 1993. Initiation of enzyme-altered foci by the synthetic steroid cyproterone acetate in rat liver foci bioassay. Carcinogenesis, 14: 1229-1231.
47. Rouse, E.F., C.J. Coppenger and P.R. Barnes, 1977. The effect of an androgen inhibitor on behavior and testicular morphology in the stickleback *Gasterosteus aculeatus*. Horm. Behav., 9: 8-18.
48. Frenkel, A., R. Abuhasira, Y. Bichovsky, A. Bukhin and V. Novack *et al*., 2021. Examination of the association of steroids with fluid accumulation in critically ill patients, considering the possibility of biases. Sci. Rep., Vol. 11. 10.1038/s41598-021-85172-y.