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Research article

Long-term impacts of Newcastle disease virus infection on fertility and clutch viability in racing pigeons (*Columba livia domestica*).

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ABSTRACT

This study investigated the impact of Newcastle disease virus (NDV) on the reproductive efficiency of racing pigeons (*Columba livia domestica*). A total of 160 pigeons (1–3 years old, 400–550 g) were selected based on clinical signs of NDV, confirmed by RT-PCR. Birds were divided into four groups: G1 (control, 20 healthy females and 20 healthy males), G2 (20 recovered females and 20 recovered males), G3 (20 healthy females and 20 recovered males), and G4 (20 recovered females and 20 healthy males). Behavioral analysis revealed significant differences in reproductive parameters. Hatchability rates were 95%, 20%, 65%, and 55% for G1, G2, G3, and G4, respectively, with incubation periods of 18 ± 1.2 to 18 ± 2.1 days. Squab acceptability among parents remained stable (~75% in all groups), and squab survival and flight ability were unaffected. However, parental care ability was significantly lower in recovered groups (55% in G2 vs. 100% in G1). Mating efficiency in G1 (100%) was significantly higher than in recovered pigeons (30%–85%). Histopathological examination of the reproductive organs in recovered pigeons revealed degeneration of ovarian follicles, including shrinkage, necrosis, and granulosa and theca cell disintegration. Testicular tissue exhibited seminiferous tubule collapse and detachment of spermatogonia from the basement membrane, compromising sperm production. These findings indicate that NDV infection causes long-term histopathological damage to reproductive tissues, reducing fertility in recovered pigeons. Further research is needed to explore potential therapeutic strategies to mitigate these effects. diseases.

Keywords: Newcastle disease, reproductive performance, racing pigeons.

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1. INTRODUCTION

This species of pigeon is exposed to many infections that affect its reproductive efficiency and limit the transition of the desirable genetic characteristics to its squabs, and the most important of these infections are viral diseases such as the Newcastle disease virus. The pigeons (*Columba livia domestica*) are domesticated birds kept as hobbies [1]. Because they are subjected to stress when flying over large distances during races, they require specific care before participating in races [2].

Several severe neurological symptoms, including paralysis of the legs and wings, torticollis, head and neck spasms, and circling [8]. Vaccinate healthy pigeons against NDV according to scientific programs to keep them from threatening extinction [9,10].

The study aims to benefit from recovered racing pigeons from NDV, determine the extent of investment from relative fertility performance, and identify the impacts of NDV on ovarian and tes-

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ticular tissue. From that, it becomes clear the highly important protection they provide for rare and costly birds that are at risk.

2. MATERIALS AND METHODS

2.1. Experimental design

The pigeons were housed in cages measuring **50 cm × 40 cm × 40 cm**, ensuring adequate space for movement and reducing stress. The cages were made of **stainless steel**, and each housed **two** pigeons to maintain appropriate stocking density. A total of **160 pigeons** were distributed across **80 cages**. The environment was controlled with a temperature of **22 ± 2°C**, relative humidity of **50–60%**, and **a 12-hour light/dark cycle** to support optimal health conditions. Food and water were provided ad libitum, and routine cleaning and disinfection were conducted to maintain hygiene [11]. This study was conducted on 160 racing pigeons (*Columba livia domestica*) collected from owners in the different quarters of Al-Qadisiyah province (south of Iraq). The pigeons' ages were [1-3] years and (400-550) gm in weight, which were determined by pre-examined with RT-PCR, specific oligonucleotide primers, and previous clinical indications of NDV nerve forms. Involved 80 healthy pigeons (40 females and 40 males) and 80 pigeons (40 females and 40 males) that were after recovering from the NDV. Pigeons were split into four groups: G1 consisted of 20 females and 20 males in good health as a control group; G2 consisted of 20 recovered females and 20 recovered males; G3 consisted of 20 healthy females and 20 recovered males; and G4 consisted of 20 recovered females and 20 healthy males. Gaging with male and female in each box, with sanitary, environmental conditions, and imbalanced rations.

The following criteria were monitored till breeding: hatchability ratio, incubation period, acceptability of parents for strange squabs, safety ratio, squabs' ability to fly, the ability of parenthood for squabs, and efficacy of mating.

2.2. Real Time PCR

To detect Newcastle Disease Virus (NDV) in pigeons, cloacal swabs were collected and placed in 1–2 mL viral transport medium (VTM) containing antibiotics (100 U/mL penicillin and 100 µg/mL streptomycin) to prevent bacterial contamination. RNA extraction was performed using a commercial kit such as TRIzol reagent, where 1 mL of TRIzol was added per 100 mg of tissue or 200 µL of swab supernatant. Chloroform was added in a 1:5 ratio (200 µL per 1 mL TRIzol) to separate the RNA in the aqueous phase, followed by isopropanol (0.5 mL per 1 mL TRIzol) for RNA precipitation. The RNA pellet was washed with 75% ethanol and resuspended in 30–50 µL RNase-free water. The extracted RNA was subjected to reverse transcription using 2 µg of RNA in a 25 µL reaction mixture containing 1X RT buffer, 0.5 mM dNTP mix, 0.5 µM random hexamers or oligo(dT) primers, 10 U RNase inhibitor, and 200 U M-MLV reverse transcriptase. The reaction was incubated at 42°C for 60 minutes, followed by enzyme inactivation at 70°C for 10 minutes. PCR amplification was carried out in a 25 µL reaction mix containing 1X PCR buffer (1.5 mM $MgCl_2$), 0.2 mM dNTP mix, 0.5 µM specific primers for NDV, 1 U Taq DNA polymerase, and 1–2 µL cDNA template. The primer set for the M gene included the forward primer **M+4100 (5′-AGTGATGTGCTCGGACCTTC-3′)** and the reverse primer **M−4220 (5′-CCTGAGGAGAGGCATTTGCTA-3′)**, which amplify a 151bp fragment of the M gene. The PCR conditions included an initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation (95°C for 30 sec), annealing (50–55°C for 30 sec), and extension (72°C for 45 sec), with a final extension at 72°C for 5 min. PCR products were separated using 1.5–2% agarose gel electrophoresis in 1X TAE buffer, stained with 1X SYBR Safe DNA stain, and visualized under UV light [12].

2.3. Histopathological Examination

Ovarian and testicular tissues were subjected to histopathological analysis by standard histological procedures [13). To preserve their structural integrity, the collected tissue samples were fixed in 10% neutral buffered formalin for 24–48 hours. Following fixation, the tissues were processed using a standard paraffin-embedding technique and sectioned into 4–5 µm thick slices using a microtome. These sections were stained with Hematoxylin and Eosin (H&E) to facilitate histopathological examination under a light microscope.

2.4. Statistical Analysis

Statistical significance was examined using Chi-square for ratios, IBM Statistical Package for the Social Sciences (SPSS.) version 22 (International Business Machines Corp., Armonk, NY, USA). The statistical significance criterion for results was $p \leq 0.01$ [14].

3. RESULTS AND DISCUSSION

The results indicated that the hatchability ratio was the highest in the G1 group compared to the other groups, with rates in the G1, G2, G3, and G4 groups being (95, 20, 65, and 55) %, respectively. This could be because healthy pigeons can reproduce, which aligns with data from [15] showing that 87% of pigeons hatch. Moreover, the hatching rate was 77% using the explanation from [16] as a guide. Moreover, this result supports the explanation in [17] that the low fertility rate is caused by the impact of testicular and ovarian tissue in ND. It also supports the findings in [18] that NDV affects reproductive performance and replicates in the ovaries and oviducts, causing severe inflammation and apoptosis, leading to decreased egg production.

The incubation period in all groups recorded means were (18±1.2, 18±2.1, 18±1.5& 18±1.3) days respectively. The findings of [15] showing pigeons require 17–19 days to incubate align with this. The present investigation's findings align with those of [19], which demonstrated that the pigeons' incubation period was 17 days, and with [20], which clarified that the pigeons' incubation period was 18–19 days.

Although the data indicated that the highest percentage of parents were accepting of strange squabs were (75, 76, 75, and 75)% respectively. Parents may have an innate tendency to welcome unfamiliar squabs into pigeon nests. This is similar to [21] assertion that squabs can be moved between pigeon nests if one or both pigeon parents pass away, that the foster style has an impact on the squab's behavior and outgrowth performance, and that the study's findings support [22] assertion that if both parents pass away, the responsibility for caring for the squabs can be passed on to foster parents.

The study's results regarding squab safety were clinically normal in all study groups, and the ability of squabs to fly was normal in all groups in that order. Moreover, the ability of parenthood for squabs was (100, 55, 90, 90) %, respectively. The ability of healthy parents to provide squabs with the nutrition they need to stay healthy, fly, and compete in races. This agrees with [22] explains that the pigeon parents are responsible for providing the squabs with food and care to ensure their health. This could be because healthy pigeons can naturally feed their young. These results support [21,23] and explain how pigeons are different from other domestic fowls in their mating and brooding habits; youngster squabs completely depend on their parents for sustenance and well-being.

The current study's mating ability results were (100, 30, 75, 85) % respectively. The healthy pigeons' ability to mate was significantly higher than that of the recovered pigeons, possibly because the neurological signs of sequels ND prevent the recovered pigeons from completing the mating process normally. The results of the current study support the findings of [8] that birds recovered from ND experience neurological symptoms, such as a loss of control over their movement and balance, and [24] that the virus could replicate in multiple tissues but that the brain was the most affected organ. This finding is consistent with that of [25] that the neurological signs could be attributed to the severe inflammation responses induced by PPMV-1; in the brains of infected pigeons. (4) also stated that ND causes neurological symptoms (Table 1).

Table 1. The observed behaviors parameters in all experimental groups.

Parameters							
Group	**HR (%)**	**IP (days)**	**APSS (%)**	**SS**	**ASF**	**APS (%)**	**EM (%)**
G1	95 [a]	18± 1.2	75 [a]	Clinically normal	Normal	100 [a]	100 [a]
G2	20 [c]	18± 2.1	76 [a]	Clinically normal	Normal	55 [b]	30 [c]
G3	65 [b]	18± 1.5	75 [a]	Clinically normal	Normal	90 [a]	75 [b]
G4	55 [b]	18± 1.3	75 [a]	Clinically normal	Normal	90 [a]	85 [a b]
Calculated X^2	46.85		0.098			34.35	27.33
Calculated P	<0.001*		0.992			<0.001*	<0.0001*

* Different letters mean significant variances (P≤0.01). Keys: *HR (%)= Hatchability ratio (%) * IP (days)= Incubation period * Acceptability of parents for Strange squabs (%)=Acceptability of parents for Strange squabs * SS= Squabs safety * ASF= Ability of squabs for fly * APS (%)= Ability of squabs for fly * EM (%)- Ability of squabs for fly.

3.1. Results of RT-PCR

The pigeons were diagnosed with Newcastle disease (NDV) using RT-PCR test. RNA was extracted from the collected samples and subjected to RT-PCR using specific oligonucleotide primers designed for this purpose, targeting the (M) gene of NDV. The amplification results confirmed the presence of NDV, with the obtained PCR product size measuring 151 bp (Figure 1), which aligns with the expected fragment size for NDV detection based on the primers specifically designed for this assay. This molecular diagnostic approach provided a highly specific and sensitive confirmation of NDV infection in the studied pigeons, supporting further histopathological and reproductive assessments.

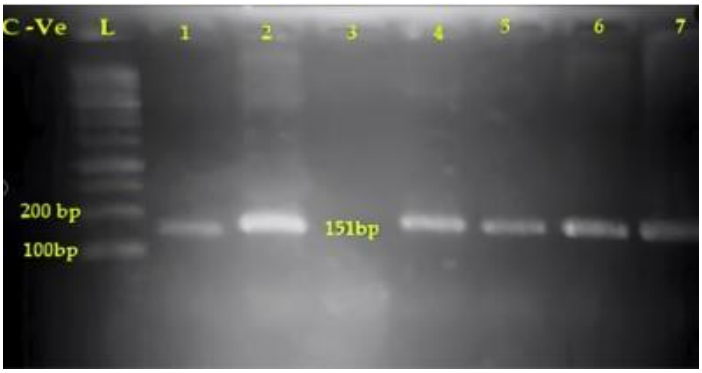

Fig. 1. RT-PCR detection of NDV targeting the M gene (151bp). Lanes: **L**: 1500 bp DNA ladder (100 bp increments), C-Ve: Negative control, 1-7: Tested samples (1, 2, 4, 5, 6, and 7 positives; 3 negative).

3.2. Results of Histopathological Findings

Healthy pigeons' ovarian histology showed follicles at various developmental stages and a normal architecture (Figure 2a-G1). Ovarian follicles had a well-preserved structural integrity, with intact theca cells and an ordered granulosa layer. The ooplasm showed no symptoms of degradation and seemed uniform, suggesting a healthy reproductive system.

Seminiferous tubules of healthy male pigeons showed a well-organized structure with all developmental stages of spermatogenesis, according to histological analysis. Along with Sertoli cells, which are crucial for spermatogenesis, spermatogonia, primary spermatocytes, secondary spermatocytes, and spermatids were all readily visible. A fully functional spermatogenic process that enables effective sperm generation is suggested by the presence of these cells in normal proportions (Figure 2b-G1).

The control group's normal ovarian and testicular histological structure serves as a benchmark for comparison with other groups and validates the lack of pathological alterations. Seminiferous tubules and well-preserved ovarian follicles show ideal reproductive function. These results are consistent with earlier research showing the normal reproductive histology of healthy birds, where ordered spermatogenesis and intact follicular architecture are suggestive of appropriate fertility potential (26).

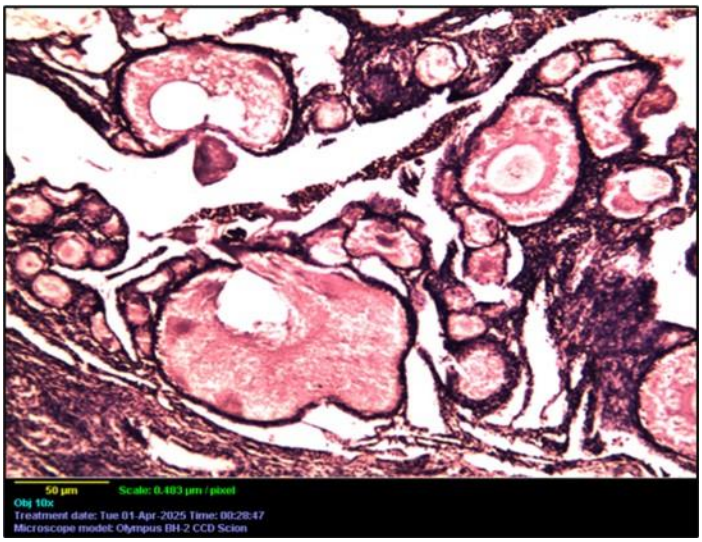

Fig. 2a-G1. Ovary of a healthy pigeon (control group) showing a normal structure with follicles at different developmental stages. H & E, 10 X.

Degenerative alterations were observed in the ovarian follicles of recovered pigeons (Figure 3a-G2). These included granulosa and theca cell shrinkage, necrosis, and occasionally total disintegration. These alterations point to impaired follicular function, most likely brought on by prior ovarian tissue pathological states. Although the almost normal structure improves, lingering follicular integrity loss suggests possible long-term reproductive impairment.

Likewise, degenerative changes were seen in the seminiferous tubules of retrieved male pigeons. Spermatogonia and the basement membrane separated, which could impair their capacity to multiply and develop into mature sperm (Figure 3b-G2).

Histopathological alterations found in the testicular and ovarian tissues of pigeons that were retrieved suggest that a prior NDV infection might have resulted in long-term reproductive harm. Impaired folliculogenesis is suggested by ovarian changes, which include cytoplasmic vacuolization, follicular atresia, and degeneration of the granulosa and theca cells.

NDV-induced reproductive failure in birds has been documented in earlier research, where ovarian shrinkage and follicular degeneration were caused by virus-mediated inflammation and vascular impairment (26). According to research showing that NDV interferes with spermatogenesis and breaks down the blood-testis barrier, the seminiferous tubules also showed degenerative changes, such as spermatogenic cell separation and basement membrane vacuolation (17).

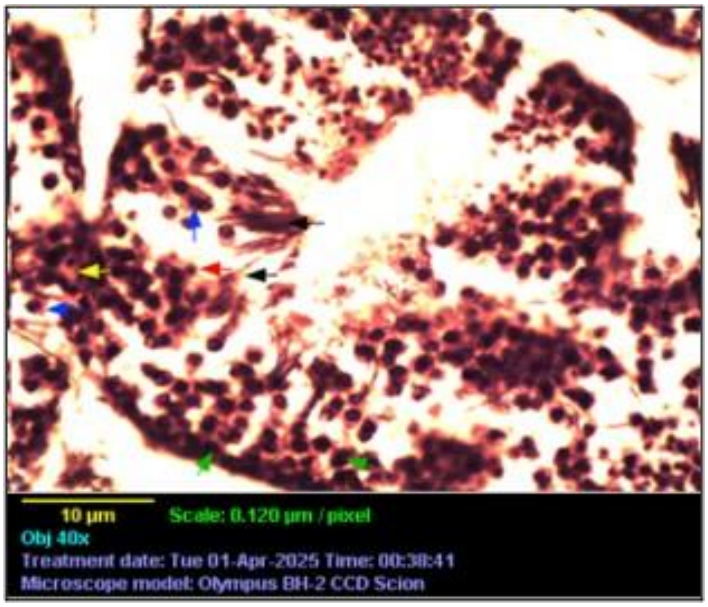

Fig. 2b-G1. Seminiferous tubules of a healthy male pigeon (control group) showing a normal structure with different developmental stages of spermatogenesis, including spermatogonia (green arrows), primary spermatocytes (blue arrows), secondary spermatocytes (red arrow), spermatids (black arrows), also Sertoli cells were noticed (yellow arrow). H&E, 40X.

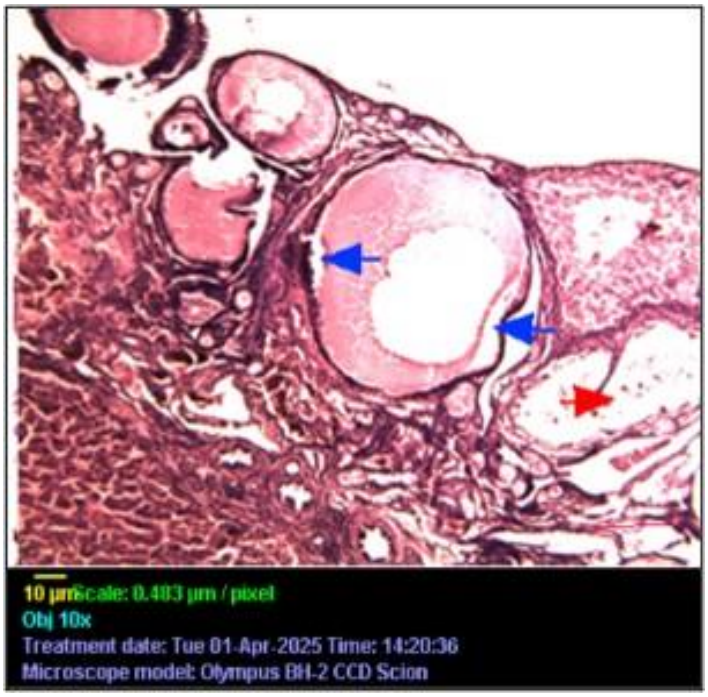

Fig. 3a-G2. Ovary of a recovered pigeon displaying nearly normal structures; however, ovarian follicles show signs of degeneration, including shrinkage, necrosis (red arrow), or disintegration of granulosa and theca cells (blue arrows). H&E, 10x.

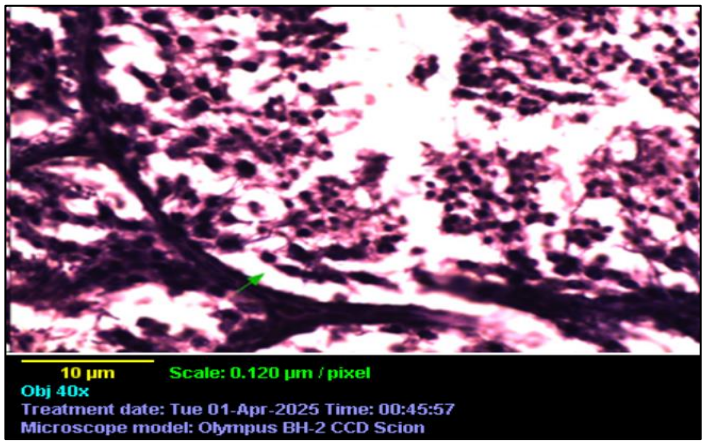

Fig. 3b-G2. Semineferous tubules of a recovered male pigeon displaying nearly normal structures; however, testis show signs of degeneration, including detachment between spermatogonia and basement membrane (green arrow), decrease number of spermatogenesis cells were noticed. H&E, 40x.

Healthy females mated with recovered males had atretic follicles with a broken perivitelline membrane in their ovarian histology (Figure 4a-G3). Follicle atresia was indicated by the leakage of granulosa layer contents into the follicular cytoplasm as a result of this rupture. Multifocal regions of rarefication and vacuolization were also present in the ooplasm, suggesting cellular stress and compromised follicular growth.

The male pigeons that were retrieved showed more noticeable degenerative alterations in their seminiferous tubules. Spermatocytes were seen to separate from the basement membrane, and there was vacuolation in the basement membrane (Figure 4b-G3). The histological alterations seen in the testicles and ovaries imply that previous NDV infection in males may have indirectly affected their partners. When healthy females mate with recovered males, atretic follicles and disruption to the perivitelline membrane may suggest that the immune system or virus-mediated hormonal imbalances influence folliculogenesis. These findings support previous reports that NDV infection can result in prolonged reproductive impairment (27,28). Similarly, NDV-induced testicular injury is further confirmed by degenerative alterations in seminiferous tubules, such as vacuolation and spermatogenic cell detachment (29).

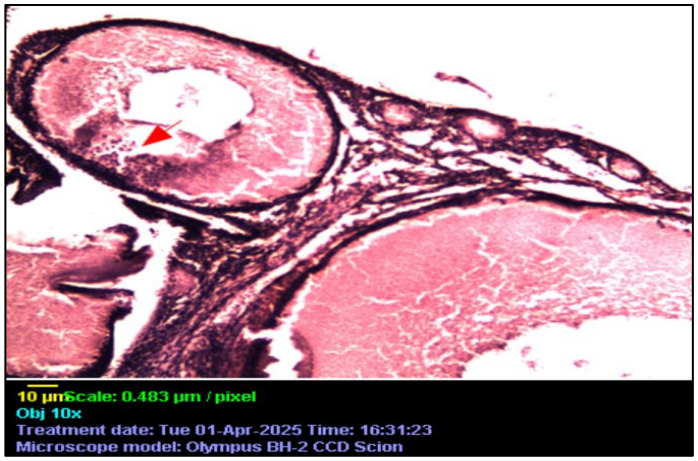

Fig 4a-G3. Ovary of a healthy pigeon showing atretic follicles with a damaged perivitelline membrane, leading to the release of granulosa layer contents into the follicular cytoplasm. The ooplasm has lost its homogenous appearance due to multifocal areas of rarefication and vacuolization (arrow). H&E, 40X.

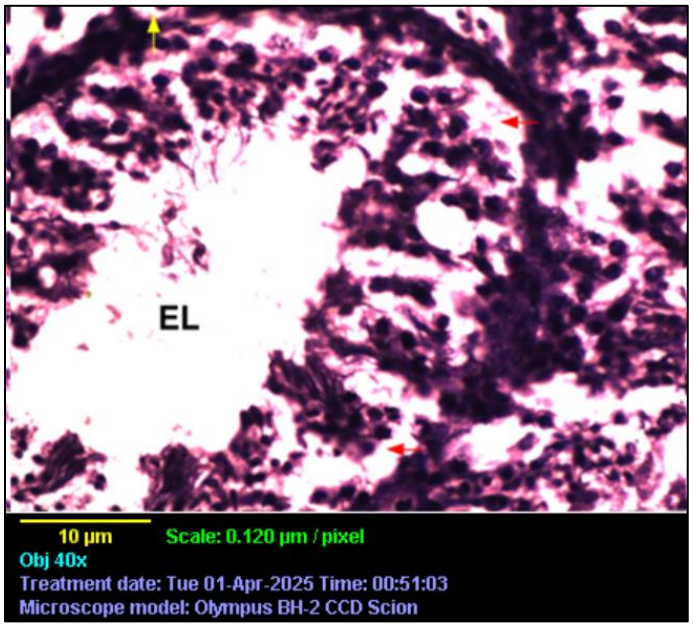

Fig. 4b-G3. Semineferous tubules of a recovered male pigeon showing vacuolation in the basement membrane (yellow arrow), detachment between spermatocytes and basement membrane (red arrows), diminished number of spermatogenic cells, and empty lumen (EL) were evident. H&E, 40x.

The ovarian histology of recovered females matched with healthy males (Figure 5a-G4) also revealed atretic follicles with a damaged perivitelline membrane. The presence of rarefied and vacuolated ooplasm and the discharge of granulosa cell contents into the follicular cytoplasm were noticeable.

In the seminiferous tubules of healthy male pigeons, a significant accumulation of different types of spermatogenic cells within the lumen was observed (Figure 5b-G4), reducing fertility and reproductive success.

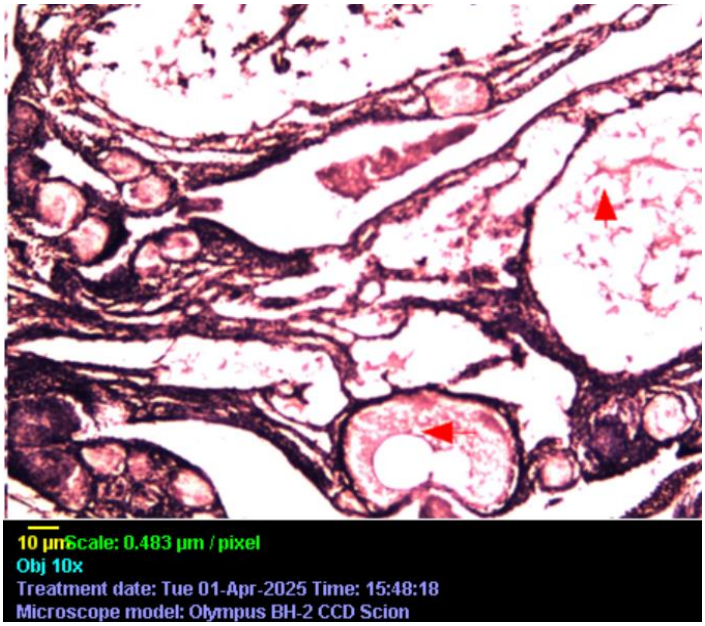

Fig 5a-G4. Ovary of recovered pigeon showing atretic follicles with a damaged perivitelline membrane, leading to the release of granulosa layer contents into the follicular cytoplasm. The ooplasm has lost its homogenous appearance due to multifocal areas of rarefication and vacuolization (arrows). H&E, 10x.

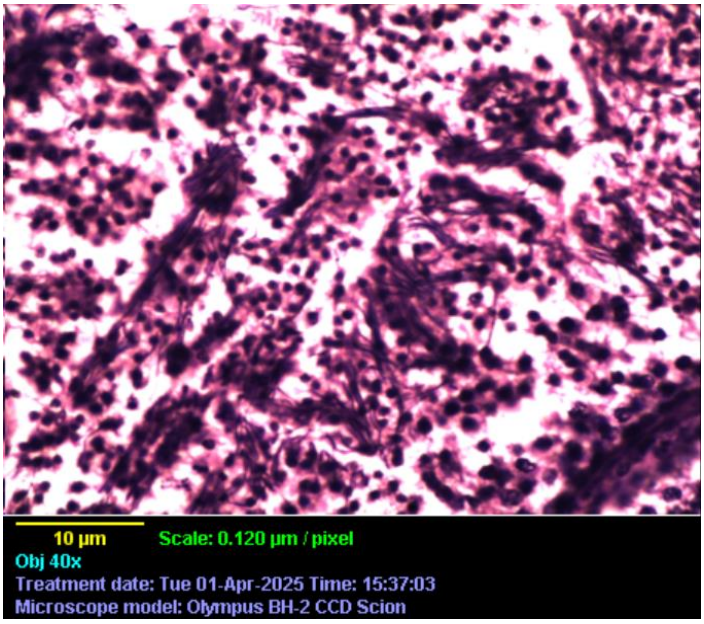

Fig. 5b-G4. Seminiferous tubules of healthy male pigeon showing filled lumen with different types of spermatogenesis cells. H&E, 40x.

Even in those that recover, the results in this group show that NDV infection causes long-lasting harm to the reproductive tissues. In females who have already been infected, the persistence of atretic follicles and disturbed perivitelline membranes indicates long-term changes in ovarian function. Similar to this, the buildup of spermatogenic cells in the seminiferous tubule lumen indicates impaired sperm maturation and transport, which is a known side effect of testicular inflammation brought on by viruses (30). These findings are consistent with research showing that NDV infection can harm birds' reproductive systems over time, thereby lowering fertility and success rates.

Nonetheless, some research indicates that reproductive structures may recover to some extent after NDV infection, especially if birds get immunomodulatory therapies. (31) showed that antioxidant supplements could increase spermatogenesis and promote follicular regeneration by reducing the damage to the ovary and testicles. This contrasts our results, which showed recovered pigeons had chronic structural damage, suggesting that recovery dynamics may vary depending on the species.

4. Conclusions

This study demonstrates that even after clinical recovery, Newcastle Disease Virus (NDV) infection causes persistent histological damage in the reproductive organs of pigeons. Ovarian alterations suggest impaired folliculogenesis and potential fertility reduction, including cytoplasmic vacuolization, follicular atresia, and degeneration of granulosa and theca cells. Similarly, testicular changes, such as basement membrane vacuolation, spermatogenic cell separation, and halted sperm production, indicate NDV-induced testicular dysfunction. These findings highlight the long-term consequences of NDV on avian reproductive health and underscore the necessity of further research into therapeutic strategies to mitigate reproductive damage. Future studies should explore immunomodulatory and antioxidant therapies to support recovery and improve reproductive outcomes in NDV-affected populations.

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Conflict of interest

The authors declare that they have no conflict of interest.

Ethical Approval & Animal ethics and care

The current study followed the National Research Council's guidelines for the care and use of laboratory animals. The University of Al-Qadisiyah, College of Veterinary Medicine's Ethical Council approved this experiment.

The care and use of animals were conducted by all relevant national, international, and/or institutional guidelines. Every procedure used in animal-based research complied with the ethical guidelines set forth by the organization or practice conducting the study.

In the materials and method section, we explained the ethical status of the animals used in our investigation and the references we relied on.

Author contributions

AHJ: Investigation; Project administration; Resources; Supervision; Validation; Roles/Writing - original draft; and Writing - review & editing, Resources; Supervision; Validation.

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