Iraqi Journal of Veterinary Sciences

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Effects of varying electroejaculation voltages on semen quality, reproductive efficiency, and testicular histology in young and old roosters

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Article information

Article history:
Received 27 May 2025
Accepted 08 August 2025
Published 10 September 2025

Keywords:
Rooster
Electrical ejaculation
Semen
Stress
Age

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Abstract

This study comprehensively evaluated effects of varying electroejaculation voltages on semen quality, reproductive efficiency, and testicular histology in young (YG) and old (OG) local roosters, while young roosters exhibited peak total, progressive motility, and velocity metrics at medium voltage, according to sperm motility and kinematic traits. In both groups, high voltage significantly ($P \leq 0.05$) reduced motility, with the decline proving more noticeable in old roosters. Old roosters achieved their peak at low voltage, but young roosters exhibited a similar pattern of peaking at medium voltage for semen volume, sperm concentration, and viability. Both groups' semen quality declined and sperm abnormalities significantly increased with high-voltage stimulation, particularly among old roosters. The Gonadosomatic index of young roosters peaked at medium voltage and declined at higher voltages, but old roosters displayed less noticeable alterations. The oxidative stress indicators malondialdehyde were lowest at medium voltage in young roosters and at low voltage in old roosters. In contrast, plasma membrane integrity and testosterone concentrations reflected this pattern, with young roosters obtaining the best reproductive outcomes at medium voltage, and old roosters exhibited maximal at low voltage. Both age groups showed significant impairment at high voltage, fertility and hatchability rates supported patterns in semen quality. Old roosters displayed early degenerative alterations even at low to medium voltage, but the young roosters at medium voltage had well-preserved seminiferous tubules according to histological analysis; seminiferous architecture was disrupted, and mature spermatozoa were lost, resulting in severe testicular degeneration in both age groups caused by high voltage. In conclusion, indicate findings underscore the crucial importance of tailoring electroejaculation voltage according to rooster age when maximize semen quality and reproductive efficiency. Low voltage is better for old roosters, while medium voltage is recommended for young roosters. Excessive voltage is deleterious regardless of age.

DOI: 10.33899/ijvs.2025.160813.4329,

Introduction

The scientific and practical challenges facing the success of reproductive efficiency strategies are those facing artificial insemination programs aimed at promoting desirable genetic strains in the poultry industry, given that these programs rely primarily on semen quality. This quality is affected by multiple factors, including the rooster's health, nutrition, age, environmental temperature and pollution (1). Still, one of the most crucial risk factors often disregarded is the method of sample collecting itself. Inappropriate techniques might harm the reproductive system of the bird or contaminate the sample with collection waste such as uric acid and feces (2). This is

especially true for species where a lack of domestication makes traditional methods of such manual massage (abdominal massage) difficult to employ (3). This demands bird training as well as appropriate pressure or stimulation application. Any flaw in the protocol might lead to the failure or loss of quality of the ejaculation, therefore influencing artificial insemination efficiency and sperm vitality. From this perspective, the need to develop effective and safe gathering techniques is stressed. Since it lets semen be retrieved from untrained roosters, electroejaculation has grown to be a significant and interesting alternative. Electroejaculation (electrical stimulation) procedure is a relatively veterinary method for collecting semen from male mammals (4,5). Its application has been extended to some bird species, but to a limited extent, particularly in roosters, quails, pheasants, ducks, and geese, in addition to parrots, wild and aquatic birds (6,7). the composition and source of seminal fluid in avian species show quite great anatomical and physiological variation. Unlike mammalian semen, in which accessory sex glands mostly contribute, the seminal fluid in birds may originate from many anatomical sites such the paracloacal vascular body, lymphatic folds, dorsal proctodeal glands, the ejaculatory groove, or tissues near the papilla of the ductus deferens, with the exact source varying depending on the species (8,9). Therefore, the unique structure and physiology of the avian reproductive tract, as well as the specific traits of seminal fluid components such as transparent fluid, highlight the need for customized methods for avian semen collecting, evaluation, and employment (10). The scientific idea of the technique is based on inserting a straight probe through the cloacal opening and adjusting it to a specific frequency and voltage sufficient to excite the pelvic nerves without causing damage to the bird (11). The importance of this method is in its capacity to standardize collecting conditions and reduce variation between samples received. This results in an involuntary reflex ejaculation response without the need to use mechanical methods. Semen is collected in a manner free of contamination by impurities such as feces or urinary salts that may appear when using manual methods such as abdominal massage or behavioral stimulation (12). Advanced analysis techniques such as Computer-Assisted Semen Analysis (CASA) enable a more accurate assessment of the effect of electrical stimulation on ejaculation quality in terms of actual reproductive efficiency, not just morphological criteria (13,14). Although this approach has benefits, the effective use of electroejaculation in roosters depends on closely monitoring and changing electrical voltage levels. When determining the ideal voltage intensity, one must give great consideration since applying improper voltage levels could stress the bird physically. Furthermore, be timing frequency, and pulse strength to provide effective stimulation of the bird's reproductive neurons without causing excessive tissue damage or stress. Studies have shown that above the recommended voltage range, unfavorable physiological consequences, including increased cortisol levels, more sperm abnormalities, and a general decline in semen quality. Whereas, following exact guidelines depending on suitable voltage distribution with suitable pulses and rest intervals helps to improve sperm vitality and focus. In advanced artificial insemination schemes, this has been shown to improve fertilization and hatching rates (12,15). Developing better procedures for semen collecting via electrical stimulation depends on an understanding of the link between electrical potential and semen quality (16).

The present work sought to find the ideal level that would greatly affect semen quality assessment and consequent reproductive results through electroejaculation applied with varying electrical voltage levels for two age groups.

Materials and methods

Ethical approve

The Ethical Committee of the University of Mosul approved this study dated 26/04/2020, issued UM.VET.2020.05.

Procurement and management of experimental animals

Sixty healthy adult local breeder roosters of two age groups (young:11-15 month; old:24-28 months) were purchased from poultry farms in Al-Hamdaniya District, Iraq. Roosters were randomly divided into six groups (n =10 roosters/group) with three voltage treatments per age group. They were housed in separate indoor cages (1.5×1.5×2 m) (length×width× height) in uniform management conditions of photoperiod exposed to a 16 h-light/8 h-dark and temperature (18-22°C) throughout the experiment with feeding and drinking water *ad libitum* and they were allowed to acclimatize to the environment for one week before the commencement of the experiment, None of the roosters had prior experience with semen collection.

Electroejaculation protocols

Roosters were eliminated food 12 hours before collection to avoid contamination of the semen. Semen was collected twice weekly using an electrostimulator device (Harvard Apparatus, Ser. No K022570, England) with a lubricated cloacal probe (6 cm length, 0.5 cm diameter). Three voltage levels were tested: Low (3-9 V): 5-7 pulses of 2-3 s each, Medium (10-17 V): 5-8 pulses of 2-3 s each and High (18-25 V): 3-5 pulses of 2-3 s each. A 1-minute rest interval was maintained between pulses. Ejaculates were collected directly from the phallus to minimize contamination (17,18). A single ejaculate of semen from these protocols was collected from each of the individual roosters twice a week for six weeks. Three replicates of

semen samples were collected for evaluation of each rooster were evaluated for quality.

Volume and color

The ejaculate volume of the fresh semen was recorded directly from graduated centrifuge tubes immediately after collection by the naked eye. The color was visually evaluated immediately after collection and graded into 4-point scale based on opacity and whiteness before further laboratory analyses were performed: 1(watery or translucent), 2 (opalescent), 3 (milky white) and 4(creamy white). The pH of the semen sample of each rooster was measured using a pH test strip and ranged from 6.4 to 8, Merck special indicator paper (19).

Motility and kinematic parameters

Total motility, velocity and other kinematic characteristics of sperm movement were recorded by means of Computer Assay Semen Analysis (CASA; HTM IVOS v. 12; Hamilton-Thorne, Beverly, USA)., semen was diluted with ratio of 1:100 (semen extender) using modified Ringer's solution (sodium chloride: 68 g, potassium chloride: 17.33 g, calcium chloride: 6.42 g, magnesium sulphate: 2.5 g, sodium bicarbonate: 24.5 g into 1000 mL of distilled water ; pH and Osmolarity were set at 7.1 and 310 mOsm/kg, respectively. diluted semen (10 µl) was applied to a 37 °C pre-warmed 20 µm Leja® 8-chamber slide (Leja Products B.V., Nieuw-Vennep, The Netherlands) enclosed by a coverslip (22×22 mm) for analyzing the motility of 500 cells, the following motion characteristics of sperm : The proportions of total motile (MOT, %), and progressive motile sperm (MP, %), Curvilinear Velocity (VCL, µm/s), Straight-Line Velocity (VSL, µm/s), Average Path Velocity (VAP, µm/s), Linearity (LIN = VSL/VCL, %), Straightness (STR = VSL/VAP, %), Amplitude of Lateral Head Displacement (ALH, µm) and Beat Cross Frequency (BCF, Hz). were specified by assessment of at least three randomly selected microscopic fields (200-300 spermatozoa) were determined for each sample.

Sperm concentration

Determined by using an improved Neubauer hemocytometer technique (20). A drop (20 µl) of semen was mixed with 3980 µl of 2% NaCl eosin at the dilution rate of 1:200, and then a droplet of semen was placed on the edge of cover slip and spermatozoa were allowed to settle for two minutes on hemocytometer chamber the loaded chamber was then placed on the microscope at a magnification of x400. The spermatozoa were then counted in five medium squares; four at corners and one at the center of 25 medium squares. The number of spermatozoa in five medium squares were counted and average number was recorded. The concentration of the spermatozoa per ml of semen was calculated by multiplying the average number of spermatozoa in medium five squares with dilution factor. The concentration was calculated using the formula, Sperm concentration expressed as billion (10^9) per ml = 50, 000 x N x D, Where N = Number of spermatozoa counted, D = Dilution rate.

Sperm viability

Eosin-nigrosine stain was used to evaluate sperm (21). The stain was prepared by dissolving 0.85 gm of Eosin (E. Merck, Germany), 5 gm of Nigrosine (H_2O soluble, British Drug House Ltd., England), and 1.45 gm of sodium citrate (H_2O soluble; E. Merck, India) in 50 ml of distilled water. Briefly, 10 µl of fresh semen was mixed with 20 µl of Ringer's solution. The 10 µl of eosin nigrosine stain was dropped onto a clean glass slide and mixed with 10 µl of diluted semen. Smears were prepared on a warm slide (22). The second glass slide was then used to swipe quickly, form a thin layer, and air dry. Viability was assessed by counting at least 200 sperm under phase contrast at ×1,000 magnification under a light microscope. Sperm displaying strict stain exclusion appeared white-headed and were counted as viable (unstained cells due to no penetration of eosin stain); sperm that had red or dark pink heads were counted as dead (23).

Abnormal morphology

To evaluate the morphological defects, 3 drops (10 µL) of semen samples were pipetted into 1 mL of Hancock's solution (24), which consisted of 62.5 mL formalin (37% formaldehyde), 150 mL sodium saline solution, and 500 mL double-distilled water. To detect the different features of total sperm abnormalities in the acrosome, head, midpiece, and tail, 10 µL of processed sperm was handled on a slide. The percentage of sperm abnormalities was determined by counting about 300 spermatozoa under a phase-contrast microscope.

Assessment of sperm membrane functionality

The hypo-osmotic swelling assay (HOS) was used to evaluate the plasma membrane integrity of the sperm (25) relies on resistance to loss of permeability barriers of the membrane under stress conditions induced by stretching in a hyper-osmotic medium. The HOS solution was prepared with fructose (9 g/L in distilled water) and sodium citrate (4.9 g/L in distilled water). This assay was carried out by mixing 5 µL of semen with 50 µL of a 100 mOsm/kg hypoosmotic solution and incubating at 37°C for 30 minutes. A drop of incubated solution was placed on a slide (37°C) and fixed in buffered 2% glutaraldehyde. Then, a total of two hundred sperm were evaluated by counting in at least five fields and randomly assessed under a phase contrast microscope, to determine the percentage of sperm with swollen and curved tails. Sperm with non-swollen tails were considered as nonfunctional (damaged sperm membranes allow fluid to pass across the membrane without any accumulation), and those with swollen and

coiled tails were classified as normal, having an intact plasma membrane (an undamaged sperm membrane permits passage of fluid into the cytoplasmic space, causing swelling).

Gonadosomatic index (GSI)

Is a ratio that expresses the weight of the Gonad's (right and left testis weight) as a percentage of the total body weight. It is a common measure used alongside semen quality parameters to provide a broader picture of reproductive status (23). GSI (%) = (Gonad's weight (g) ÷ Total body weight (g)) × 100. Determination testosterone concentration at end time of the experiment, An ELISA kit (EIA-1559, DRG Instruments, Germany) was used to determine it (26). The microtiter plates were coated with a monoclonal mouse antibody and used to analyze duplicate 25-µl serum and standards samples (0.2-16 ng/ml). Plates were incubated for 60 minutes after the addition of the enzyme conjugate, after which plates were cleaned and exposed to tetramethylbenzidine substrate for 20 minutes. A stop solution was employed for stopping the reaction, and an ELISA reader was utilized to quantify absorbance at 450 nm. to compute the levels of testosterone. Also, BC0025 MDA Content Assay Kit (Solarbio, China) utilized the thiobarbituric acid reactive substances (TBARS) method to measure the quantity of malondialdehyde (MDA) in serum. The reaction between MDA and thiobarbituric acid (TBA) establishes a pink chromogen that is detected spectrophotometrically at 532 nm.

Histological examination

Testis preparation for histological sectioning was obtained from six experimental groups at the conclusion of the trial. Following humane euthanasia, the left testis was meticulously excised and promptly fixed in 10% neutral buffered formalin for a duration of 24 to 48 hours. The fixed tissues from each group underwent a standard histological preparation procedure, which involved dehydration through a graded ethanol series (70%, 80%, 95%, and 100%), clearing with xylene, and embedding in paraffin wax. Microtomy yielded sections with a thickness of 5 µm. Samples were prepared on glass slides and stained with Hematoxylin and Eosin (H&E) for histological analysis using a light microscope (27).

In vivo fertility and hatchability assessment examining

Reproductive performance, artificial insemination (AI) was performed (28,29), with minor modifications to assess the fertility parameters of six experimental groups of roosters, selected based on the results of in vitro sperm evaluation. The semen obtained from 6 roosters in each group was pooled and then diluted using modified Ringer's solution, Fertility parameters were measured by inseminating ninety laying hens, 45 weeks old, with 80% egg production, which had not had previous contact with the roosters for 1 month and were candidates for artificial insemination (AI) (divided into 6 groups of n = 15 individual hens each). Artificial insemination was done twice a week with 0.4 ml of pooled semen from each group; Insemination was performed between 3:00 pm and 5:00 pm. The eggs were marked and collected up to five days after the last artificial insemination and were selected for incubation and disinfected with formaldehyde for 15 minutes according to the recommended concentration of 1.2 mL of formalin added to 0.6 g of potassium permanganate. Afterward, eggs were set in a common incubator (Qingdao Farm Lyric Agri-tech Co., China) for 18 days at a temperature of 37.7°C and a relative humidity of 75%, The fertility attributes were calculated with the following formulas: Fertility rate and early embryonic survival were measured by candling the eggs on the 7th day of incubation for each group as the percentage of fertile eggs of the total number of incubated eggs (fertile eggs/incubated eggs) Infertile eggs were broken open to confirm the absence of embryonic development. On day 18 of post-incubation, the eggs were transferred to the hatcher for the remaining 3 days of incubation, hatching rate was calculated on the 21st day of incubation. The hatchability of fertile eggs as the percentage of hatched eggs of the total number of fertile eggs (hatched eggs/fertilized eggs). The hatchability of incubated eggs as the percentage of hatched eggs of the total number of incubated eggs (hatched eggs/incubated eggs).

Statistical analysis

Data analyses were carried out with the SigmaPlot 12.5 (Systat Software Inc 2016) Values are expressed as mean with their standard error (Mean ± SE) and analyzed using a two-way analysis of variance (ANOVA), followed by Tukey's range test to determine significant differences among groups A probability level $P \leq 0.05$ was considered statistically significant.

Results

The analysis of different electroejaculation voltages has been identified through the investigation of sperm motility and kinematic parameters among young (YG) and old (OG) roosters (Table 1). Whereas older roosters demonstrated their peak motility at low voltage (80.3 % total motility, 68.0% progressive), young roosters demonstrated higher total and progressive motility at medium voltage (81.6 % total, 69.5 progressive), Both age groups indicated significant reductions in motility at high voltage; old roosters displayed a more marked reduce (total: 59.2% vs. 66.8%). Younger roosters at medium voltage typically had greater velocity values such as VCL and VSL; VCL reached 155.5 µm/s while VSL reached 130.3 µm/s in old roosters. On low voltage, elderly roosters showed rather better velocity values. Between groups at their respective

ideal voltages, linearity and straightness percentages were similar; but, at high voltage both dropped significantly. In younger roosters especially, the amplitude of lateral head motion rose with voltage; in older roosters, it stayed somewhat constant across voltages. Whereas older roosters displayed their maximum frequency at low voltage, while the younger roosters at medium voltage showed more beat cross frequency. The findings show age-dependent variations in optimal electroejaculation voltage for semen quality: young roosters gain more from medium voltage stimulation and old roosters from lower voltage; high voltage significantly harms both groups. Whereas (Table 2) older roosters indicated peak semen volume and concentration at low voltage (2.09 mL and 2.23 × 10^9/mL, respectively), younger roosters produced the highest semen volume and sperm concentration at medium voltage (2.16 mL and 2.15 × 10^9/mL, respectively). Semen pH didn't differ significantly across voltages or age groups. Indicating qualitative shifts in seminal fluid composition with age and stimulation intensity, semen color ratings were highest at medium voltage in younger roosters but peaked at low voltage in older roosters. Younger roosters at medium voltage (72.56%) had significantly greater sperm viability compared to older roosters, who demonstrated their peak viability at low voltage (66.9%. Younger roosters at medium voltage (20.7%) consistently had total sperm abnormalities that were lower than those seen in higher voltage groups; older roosters showed somewhat increased abnormalities generally. While older roosters demonstrated higher abnormalities at high voltage, specific sperm shortcomings in the mid-piece and tail areas were much reduced at medium voltage in younger roosters. Age groups or voltage treatments enjoyed no appreciable difference within head abnormalities.

Table 1: The influence of different electroejaculation voltages on sperm motility and kinematic parameters among younger (YG) and older (OG) roosters Estimated by CASA system

Variables	Age	Low Voltage	Medium Voltage	High Voltage
Total Motility (%)	YG	73.7± 1.9 ab	81.6 ± 2.2 a	66.8 ± 1.9 b
	OG	80.3 ± 2.5 a	72.0± 2.0 ab	59.2 ± 2.5 c
Progressive Motility (%)	YG	58.7± 1.7 ab	69.5 ± 2.0a	55.5 ± 1.9 b
	OG	68.0 ± 2.2a	60.3± 1.7 ab	52.0 ± 2.0 b
VCL (µm/s)	YG	130.0± 6.0 bc	155.5 ± 5.8 a	125.2± 6.0 c
	OG	148.0 ± 5.5 a	130.3 ± 5.9 bc	133.3± 5.9 b
VSL (µm/s)	YG	76.0 ± 4.0 b	95.0 ± 4.3 a	59.3± 4.0 c
	OG	93.0 ± 4.3 a	74.5± 4.7 b	60.5 ± 3.9 c
VAP (µm/s)	YG	114.5± 4.9 b	123.2 ± 5.0a	109.2 ± 5.0b
	OG	120.0 ± 4.9a	116.0± 4.5 ab	115.0 ± 5.0b
LIN (%)	YG	58.7 ± 3.8 ab	61.8 ± 4.0 a	47.3± 3.5 b
	OG	63.1 ± 4.1 a	57.4 ± 4.7 ab	45.5 ± 4.4 b
STR (%)	YG	66.7 ± 3.0 ab	77.7 ± 3.3 a	54.5 ± 2.8 b
	OG	77.5 ± 3.5 a	64.5 ± 3.3 ab	52.6 ± 3.3 b
ALH (µm)	YG	3.9 ± 0.4 c	5.0 ± 0.3 b	6.3 ± 0.3 a
	OG	4.5 ± 0.4 b	4.4 ± 0.3 bc	4.1 ± 0.3 bc
BCF (Hz)	YG	21.0 ± 1.5 b	27.0 ± 1.4 a	15.5 ± 1.4 c
	OG	24.2 ± 1.5 a	19.2 ± 1.4 b	19.5 ± 1.4 b

Values are expressed as Mean ± SEM. Different superscript letters (a, b, c) within among groups indicate significant differences $P \leq 0.05$.

The assessment of body weight and testicular parameters in younger and older roosters (Table 3) subjected to different electroejaculation voltages reveals distinct patterns for several traits, while others remain unaffected by voltage or age. Body weight remained consistent across all voltage treatments and age groups, showing no significant differences. However, testicular measurements were more responsive to voltage variation, particularly in younger roosters. In YG, both right and left testis weights were highest at medium voltage and significantly lower at high voltage, indicating that excessive stimulation may negatively affect testicular mass. Older roosters displayed a similar, though less pronounced, trend in right testis weight, with medium voltage groups showing slightly reduced values compared to low voltage. For GSI which reflects testis size relative to body weight, YG roosters achieved their highest values at medium voltage, while high voltage resulted in a significant reduction. In OG, GSI values did not differ significantly across treatments, although a modest decline was observed at higher voltage. Plasma membrane integrity (HOST %) was optimally preserved at medium voltage for YG, in contrast

to substantial reductions at high voltage; for OG, HOST did not significantly differ between low and medium voltages, but both were superior to the high voltage group. Testosterone concentrations further illustrated age- and voltage-dependent responses. In YG, testosterone peaked at medium voltage and declined at high voltage, suggesting that moderate stimulation optimizes endocrine activity, whereas excessive stimulation suppresses it. For OG, testosterone levels were lower overall, with a significant reduction observed at medium and high voltages compared to low voltage. Finally, levels of malondialdehyde (MDA), a biomarker of lipid peroxidation, were lowest in YG at medium voltage indicating reduced oxidative stress under these conditions, while both low and high voltages yielded higher MDA concentrations. In OG, the lowest MDA was observed at low voltage, but values increased with voltage, peaking at high voltage. Collectively, these findings underscore that medium voltage stimulation confers the most favorable effects on key semen quality indices in younger roosters, while older roosters tend to benefit more from low voltage, High voltage was consistently associated with detrimental effects on most semen quality traits in both age groups.

Table 2: The influence of different electroejaculation voltages on macroscopic and microscopic evaluation among younger (YG) and older (OG) roosters

Variables	Age	Low Voltage	Medium Voltage	High Voltage
Semen volume (mL)	YG	1.59 ± 0.13 b	2.16 ± 0.13 a	1.40 ± 0.10 b
	OG	2.09 ± 0.15 a	1.55 ± 0.11 b	1.43 ± 0.11b
Semen pH	YG	6.93 ± 0.15 a	7.03 ± 0.14 a	6.80 ± 0.14 a
	OG	7.22 ± 0.14 a	7.00 ± 0.18 a	6.66 ± 0.15 a
Semen color	YG	2.66 ± 0.21 b	3.40 ± 0.17 a	2.20 ± 0.19 b
	OG	3.30 ± 0.19 a	2.45 ± 0.20 b	2.33 ± 0.19 b
Sperm Concentration (10^9/mL)	YG	1.90 ±0.18 ab	2.15 ± 0.20 a	1.53 ± 0.22 bc
	OG	2.23 ± 0.22 a	1.86 ± 0.20 ab	1.47 ± 0.18 c
Viability (%)	YG	55.50±2.21 bc	72.56± 2.33 a	44.07± 2.30 c
	OG	66.9± 2.30 ab	54.7± 2.19 bc	53.20± 2.21 bc
Total Abnormalities (%)	YG	28.7 ± 2.0 b	20.7± 2.0 c	43.2 ± 1.9 a
	OG	24.1 ± 2.0 bc	24.9 ± 2.2 bc	44.7 ± 1.9 a
Head Abnormalities (%)	YG	6.0± 1.0 a	5.4 ± 0.09 a	6.0 ± 1.1 a
	OG	6.0 ± 1.1 a	5.4 ± 1.1 a	5.5 ± 1.1 a
Mid-piece Abnormalities (%)	YG	12.3 ± 1.7 ab	6.5 ± 1.5 c	19.7± 1.5 a
	OG	9.7± 2.1 bc	9.9 ± 1.5 b	20.1 ± 1.3 a
Tail Abnormalities (%)	YG	10.5 ± 1.7 ab	7.7 ± 1.7 c	17.5 ± 2.0 a
	OG	8.5 ± 1.5 bc	10.0 ± 1.5 ab	19.0 ± 2.2 a

Values are expressed as Mean ± SEM. Different superscript letters (a, b, c) within among groups indicate significant differences $P \leq 0.05$.

The findings indicate that reproductive efficiency, as measured by fertility and hatchability rates (Table 4), is highly dependent on both the age of the rooster and the applied voltage during semen collection. demonstrates notable and statistically significant patterns ($P \leq 0.05$). Among younger roosters, medium voltage consistently resulted in superior reproductive outcomes. Fertility rates reached their highest point in this group, accompanied by the best hatchability on both fertile eggs and total eggs set. In contrast, high voltage produced the poorest results for YG, with fertility dropping to 37.7%, and hatchability rates declining sharply 46.7% on fertile eggs and 17.5 % on total eggs), highlighting the negative impact of excessive stimulation. Low voltage, while outperforming high voltage, was still significantly less effective than medium voltage for most fertility measures in the younger group. For older roosters, the voltage-fertility relationship shifted. Low voltage provided the highest fertility 60.6 % and hatchability 64.2 % on fertile eggs and 38.8 % on total eggs. Elevating the voltage led to a consistent and significant decrease in all measured outcomes, with high voltage yielding the lowest fertility and hatchability percentages. Medium voltage offered intermediate performance in OG, outperforming high voltage but generally falling short of the results seen with low voltage.

Table 3: The influence of different electroejaculation voltages on Gonadosomatic index, Plasma membrane integrity, Testosterone and Malondialdehyde (MDA) among younger (YG) and older (OG) roosters

Semen features	Age	Low Voltage	Medium Voltage	High Voltage
Body weight (kg)	YG	2.33 ± 0.11	2.33 ± 0.09	2.34 ± 0.11
	OG	2.33 ± 0.09	2.31 ± 0.09	2.32 ± 0.09
Right testis weight (g)	YG	12.1± 0.17b	13.0 ± 0.19a	10.9 ± 0.22b
	OG	12.6 ± 0.20ab	11.6 ± 0.19b	11.0 ± 0.22b
Left testis weight (g)	YG	13.0 ± 0.20a	13.9 ± 0.19a	11.7 ± 0.25b
	OG	13.1± 0.20a	12.8 ± 0.20a	12.6 ± 0.22a
Gonadosomatic index (%)	YG	1.08± 0.09 ab	1.20 ± 0.07 a	0.97 ± 0.05 b
	OG	1.10± 0.09 ab	1.08 ± 0.10 ab	1.02 ± 0.07 b
Plasma membrane integrity (%)	YG	54.99± 1.1b	64.68± 1.09 a	40.1± 1.11 c
	OG	55.5± 1.00 b	47.2± 1.20 bc	41.5± 1.09c
Testosterone (ng/mL)	YG	3.77 ±0.22 ab	4.91 ± 0.21 a	3.09 ± 0.19 b
	OG	3.60 ± 0.19ab	2.97 ± 0.22 b	3.0 ± 0.22 b
MDA (nmol/mL)	YG	3.40 ± 0.20 a	2.21 ± 0.27 b	3.77 ± 0.27a
	OG	2.01 ± 0.27b	2.81 ± 0.25 ab	3.00 ± 0.20a

Values are expressed as Mean ± SEM. Different superscript letters (a, b, c) within among groups indicate significant differences P≤ 0.05.

Table 4: The influence of different electroejaculation voltages on Fertility, Hatchability on fertile and total set eggs among younger (YG) and older (OG) roosters

Fertility features	Age	Low Voltage	Medium Voltage	High Voltage
Fertility (%)	YG	44.8 ± 1.07 bc	68.2 ± 1.09 a	37.7 ± 1.13 c
	OG	60.6 ±1.11 ab	51.6 ± 1.13 b	40.2 ± 1.15 c
Hatchability on fertile eggs (%)	YG	53.8 ± 1.11 b	70.7 ± 1.22 a	46.7 ± 1.15 c
	OG	64.2 ±1.30 ab	50.8 ± 1.09 bc	46.0 ± 0.99 c
Hatchability on total eggs (%)	YG	24.2 ± 1.22 cd	49.2 ± 0.99 a	17.5 ± 1.00 d
	OG	38.8 ± 1.11 b	26.8 ± 1.09 c	18.7 ± 0.99 d

Values are expressed as Mean ± SEM. Different superscript letters (a, b, c) within among groups indicate significant differences P≤ 0.05.

Testicular histomorphology

Histological examination of testicular tissue from roosters of various ages exposed to varying electroejaculation voltages revealed distinct differences in testicular architecture and spermatogenic activity between groups. Sections of the medium-voltage younger group in (Figure 1) showed well-organized seminiferous tubules with an intact germinal epithelium and robust spermatogenesis, indicating preserved testicular function under moderate stimulation. All developmental stages of spermatogenic cells were present along with suitable interstitial tissue. Although the testicular tissue of the low-voltage older group was still exhibiting general structural integrity and active spermatogenesis, it started to show mild histological evidence of degeneration, including a minor disturbance in the arrangement of spermatogenic cells, indicating the early start of age-related testicular changes.Younger animals showed a similar positive response to low-level stimulation (Figure 2), with the low-voltage younger group exhibiting mainly normal histological characteristics and only mild, localized degradation of a small number of spermatogenic cells. The medium voltage older group exhibited mild disorganization of the seminiferous tubules and early degenerative changes in the spermatogenic population, indicating age-related sensitization of the testis to even moderate levels of stimulation. Conversely, figure 3 demonstrates that the most significant histopathological abnormalities were found in both age groups exposed to high-voltage stimulation. Younger birds exposed to high voltage exhibited severe disorder of the seminiferous tubules, rupture of germ cells, large tubular lumens, a near-complete stop of spermatogenesis with no mature spermatozoa, and significant thinning of the spermatogenic epithelium with extensive degeneration of spermatogenic cells, weakness, rupture of the germinal epithelium, and major structural collapse, these degenerative changes were far more severe in the high-voltage senior group. These results show that testicular injury exacerbated by aging and increasing voltage significantly lowers spermatogenic activity.

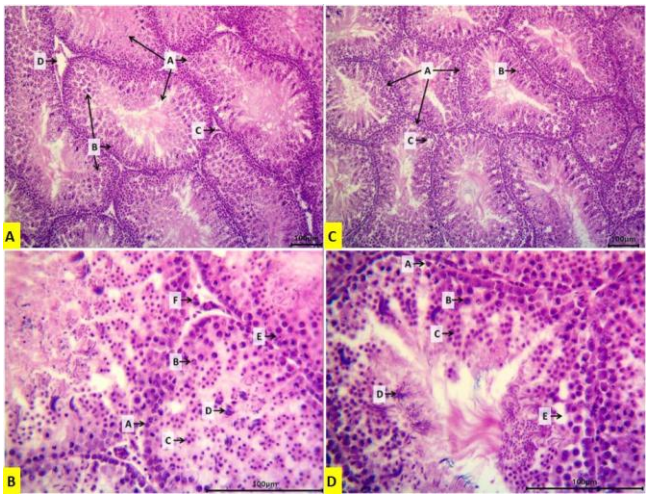

Figure 1: Histological section of rooster testis. [A&B]: Medium voltage younger group showing intact seminiferous tubules (A) with active spermatogenesis and different types of spermatogenic cells (B), interstitial tissue (C), and blood vessels (D), with intact thickness of epithelium. [C&D]: Low-voltage older group showing intact seminiferous tubules (A) with active spermatogenesis and well spermatogenic cells layer thickness (B), with mild degeneration of a few spermatogenesis cells (C), H&E stain, [A&C]:100X, [B&D]:400X.

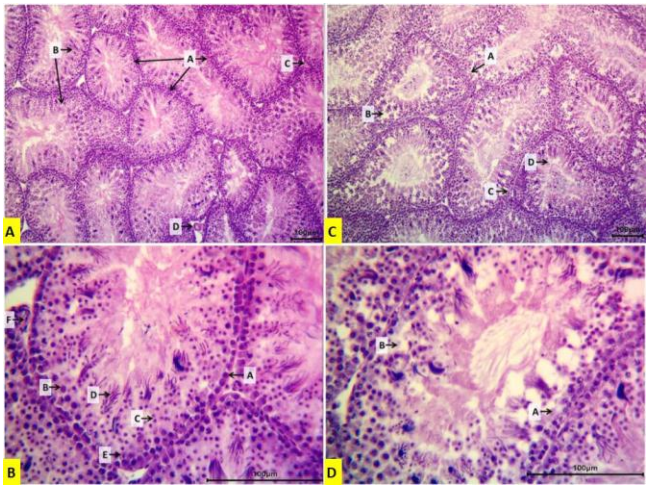

Figure 2: Histological section of rooster testis. [A&B]: Low voltage younger group showing intact seminiferous tubules (A) with mild degeneration of spermatogenic cells (B), intact interstitial tissue (C) and blood vessels (D), with intact thickness of epithelium. [C&D]: Medium voltage older group showing mild disorganization of seminiferous tubules (A) and mild degeneration of spermatogenesis cells (B). H&E stain, [A&C]:100X, [B&D]:400X.

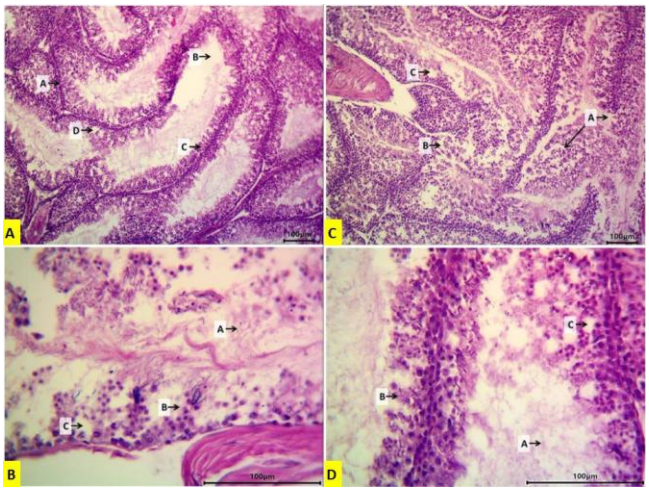

Figure 3: Histological section of rooster testis. [A&B]: High voltage younger group showing disorganization of seminiferous tubules (A) arrested spermatogenesis no spermatozoa in seminiferous tubules, wide lumen (B), decreased the spermatogenic cells layer thickness (C) with rupture of spermatogenesis cells (D). [C&D]: High voltage older group showing disorganization of seminiferous tubules (A) decreased the spermatogenic cells layer thickness (B), with rupture of spermatogenesis cells (C) H&E stain, [A&C]:100X, [B&D]:400X.

Discussion

The results of the present study provide a comprehensive evaluation of the effects of varying electroejaculation (EE) voltages on semen quality, reproductive efficiency, and testicular histology in Young (YG) and old (OG) roosters. The findings highlight significant age-dependent responses to electrical stimulation, with optimal semen quality and reproductive outcomes achieved at different voltage levels for each age group. These results align with and expand upon previous research in avian and mammalian species, emphasizing the importance of tailored EE protocols to maximize reproductive success. The analysis of sperm motility and kinematic parameters revealed that young roosters exhibited peak total and progressive motility at medium voltage (10-17 V), whereas old roosters performed best at low voltage (3-9 V). High voltage (18-25 V) significantly reduced motility in both groups, with old roosters experiencing a more pronounced decline. These findings are consistent with studies in mammals, where excessive electrical stimulation has been linked to reduced sperm motility due to oxidative stress and physical damage to sperm membranes (30,31). The superior performance of young roosters at medium voltage may reflect their greater physiological resilience and higher metabolic activity, which supports sperm motility and velocity. Conversely,

old roosters, likely experiencing age-related declines in testicular function, may be more susceptible to stress from higher voltages, as evidenced by their reduced motility at medium and high voltages. Semen volume and sperm concentration followed a similar pattern, with young roosters peaking at medium voltage and old roosters at low voltage. High voltage led to significant declines in these parameters, particularly in old roosters. These results corroborate earlier studies in birds and mammals, where moderate electrical stimulation optimized semen yield, while excessive voltage caused epithelial damage and reduced sperm production (6). The age-related differences in optimal voltage may stem from variations in testicular vascularization and nerve sensitivity, with old roosters requiring gentler stimulation to avoid tissue stress (32). The findings from (3,16) highlight the practical utility of electroejaculation (EE) as an alternative semen collection method in avian species, especially in aggressive breeds like fighting roosters. Both studies demonstrate that EE at low voltages 5-12 V offers a viable and safe alternative to conventional abdominal massage (AM) for semen collection in Siamese fighting cocks. Although its success rate in eliciting ejaculation 44.4% is lower than that of dorsal massage 100%, low-voltage EE consistently yields ejaculates with comparable overall quality and sperm motility even after prolonged incubation and demonstrates superior kinetic parameters (increased velocity, linearity and straightness) with fewer contaminants. In contrast, high-voltage EE (up to 30 V) can further enhance motility but carries significant risks, such as bleeding, which limit its practical use. Consequently, low-voltage EE is particularly well suited for untamed or aggressive birds, combining effectiveness, safety and enhanced sperm kinetics to facilitate reproductive management and breeding programs in avian species.

In present study, sperm viability and membrane integrity were highest at medium voltage in young roosters and at low voltage in old roosters, further underscoring the need for age-specific EE protocols. The decline in these parameters at high voltage aligns with research showing that excessive electrical stimulation induces lipid peroxidation and membrane damage, as reflected by elevated malondialdehyde (MDA) levels (33,34). The observed increase in sperm abnormalities at high voltage, particularly in mid-piece and tail defects, supports the hypothesis that oxidative stress and physical trauma disrupt spermatogenesis and sperm maturation. GSI and testosterone concentrations provided additional insights into the physiological responses to EE. Young roosters showed peak GSI and testosterone levels at medium voltage, suggesting that moderate stimulation enhances testicular function and endocrine activity. In contrast, old roosters exhibited minimal changes in GSI across voltages, with testosterone levels peaking at low voltage and declining at higher voltages. These findings mirror studies in aging males, where reduced Leydig cell function and testosterone production make the testes more vulnerable to stress (35,36). The decline in testosterone at high voltage may further exacerbate semen quality reductions, as testosterone is critical for spermatogenesis and sperm maturation. Oxidative stress, measured via MDA levels, was lowest at medium voltage in young roosters and at low voltage in old roosters, reinforcing the idea that optimal EE protocols minimize oxidative damage. High voltage consistently increased MDA levels in both groups, correlating with histological evidence of testicular degeneration. The severe disruption of seminiferous tubules and loss of mature spermatozoa in high-voltage groups, particularly in old roosters, aligns with studies linking oxidative stress to germ cell apoptosis and structural collapse in the testes (37,38). These histological changes likely explain the observed declines in semen quality and fertility. In present study, the fertility and hatchability results directly reflected the semen quality trends, with young roosters achieving the highest rates at medium voltage and old roosters at low voltage. High voltage severely impaired reproductive outcomes in both groups, consistent with the cumulative negative effects on sperm motility, viability, and testicular integrity. These findings emphasize that semen quality parameters are reliable predictors of fertility and hatchability, as demonstrated in prior avian studies (39,40). The current results are in agreement with research on EE in other avian species (6,41), such as quails and ducks, where moderate voltages yielded optimal semen quality (42,43). Similarly, studies in mammals have shown that excessive electrical stimulation reduces sperm quality and testicular health (44). However, this study uniquely highlights the importance of age-specific EE protocols, a factor less explored in earlier research. The differential responses of young and old roosters suggest that EE protocols must account for age-related physiological changes to maximize reproductive efficiency (45). The study focused on local roosters, and results may vary with other breeds or species. Additionally, long-term effects of repeated EE on rooster health and fertility were not assessed. Future studies could explore genetic and nutritional interactions with EE protocols to further refine semen collection techniques.

Conclusion

This study demonstrates that electroejaculation voltage must be carefully tailored to the age of roosters to optimize semen quality and reproductive efficiency. Young roosters perform best at medium voltage, while old roosters require low-voltage stimulation. High voltage is universally detrimental, causing oxidative stress, testicular damage, and reduced fertility. These findings provide a scientific basis for age-specific EE protocols, enhancing the success of poultry breeding programs.

Acknowledgment

The author is grateful to the University of Mosul, College of Veterinary Medicine for all the facilities to achieve this study.

Conflict of interest

There is no conflict of interest.

References

1. Juiputta J, Chankitisakul V, Boonkum W. Genetic strategies for enhancing rooster fertility in tropical and humid climates: Challenges and Opportunities. Animals. 2025;15(8): 1096. 10.3390/ani15081096
2. Raidal S. Laboratory diagnostics for birds. Exot Anim Lab diagnosis. 2020;429-436. 10.1002/9781119108610.ch23
3. Moscoso Piedra, A., Muñoz, M., Argudo Garzón, D., Samaniego, J., Maldonado, M., Cabrera, B., Alvarado, J. C., & Galarza, D. Comparison of characterization of fighting rooster (*Gallus gallus*) semen ejaculates recovered by electroejaculation and dorsal massage techniques. *Spermova*,2021; 11(1), 32-38. 10.18548/aspe/0009.05
4. Juri GL, Chiaraviglio M, Cardozo G. Electrostimulation is an effective and safe method for semen collection in medium-sized lizards. Theriogenology. 2018; 118:40-5. 10.1016/j.theriogenology.2018.05.035
5. Abril-Sánchez S, Freitas-de-Melo A, Giriboni J, Santiago-Moreno J, Ungerfeld R. Sperm collection by electroejaculation in small ruminants: a review on welfare problems and alternative techniques. Anim Reprod Sci. 2019; 205:1-9. 10.1016/j.anireprosci.2019.03.023
6. Frediani MH, Guida FJ V, Salgado PAB, Gonçalves DR, Blank MH, Novaes GA, & Pereira, R. J. Semen collection by electro-stimulation in a variety of bird orders. Theriogenology. 2019; 125:140-51. 10.1016/j.theriogenology.2018.10.023
7. Gee GF, Bertschinger H, Donoghue AM, Blanco J, Soley J. Reproduction in nondomestic birds: physiology, semen collection, artificial insemination and cryopreservation. Avian Poult Biol Rev. 2004;15(2):47-101. 10.3184/147020604783637435
8. Santiago-Moreno J, Blesbois E. Functional aspects of seminal plasma in bird reproduction. Int J Mol Sci. 2020;21(16): 5664. 10.3390/ijms21165664
9. Das PK, Mukherjee J, Banerjee D. Functional morphology of the male reproductive system. In: Textbook of veterinary physiology. Springer; 2023. p. 441-76. 10.1007/978-981-19-9410-4_19
10. Bezerra LGP, Oliveira REM de, Comizzoli P, Silva AR. Collection, handling, and preservation of wild bird semen: Current status, challenges, and perspectives. Biopreserv Biobank. 2024;22(3):191-210. 10.1089/bio.2023.0053
11. Singh NJ, Singh A, Singh B, Jain G. Chapter-1 Overview of the Artificial Insemination in Poultry. Vet Sci. 2020;1.
12. Bolarinwa AO. Fertility response of artificially inseminated broiler breeder hens to semen dose, insemination frequency and semen dilution. Poult Sci. 2021;100(8):101451.
13. Ayad BM. Basic semen parameters assisted by Computer-Aided sperm analysis (CASA) and their correlations with advanced semen parameters in normozoospermic men with different abstinence periods. Stellenbosch: Stellenbosch University; 2018. [available at]
14. Tanga BM, Qamar AY, Raza S, Bang S, Fang X, Yoon K, & Cho, J. Semen evaluation: Methodological advancements in sperm quality-specific fertility assessment-A review. Anim Biosci. 2021;34(8):1253. 10.5713/ab.21.0072
15. Zhang X, Chao S, Ye N, Ouyang D. Emerging trends in sperm selection: enhancing success rates in assisted reproduction. Reprod Biol Endocrinol. 2024;22(1):67. 10.1186/s12958-024-01239-1
16. Kanatiyanont N, Kornkaewrat K, Suthanmapinunt P, Pinyopummin A. Effect of semen collection techniques on semen quality and sperm motility parameters in Siamese fighting cock (Gallus gallus). Thai J Vet Med. 2012;42(4):439-45. 10.56808/2985-1130.2422
17. Naser A, Albadrany Y, Shaaban KA. Methods of Pain Assessment in Chicks as a Model. Egypt J Vet Sci. 2021;52(2):241-9. 10.21608/ejvs.2021.64605.1219
18. Tvrdá, E, Petrovičová, M, Ďuračka, M, Benko, F, Slanina, T, Galovičová, L & Kačániová, M. Short-Term Storage of Rooster Ejaculates: Sperm Quality and Bacterial Profile Differences in Selected Commercial Extenders. *Antibiotics*, 2023; *12*(8), 1284. 10.3390/antibiotics12081284
19. Lorton SP. Andrology laboratory: equipment and techniques-measurement of ejaculate volume, sperm concentration, extenders, and cryopreservation, the microscope, and sperm motility evaluation. In: Manual of Animal Andrology. CABI GB; 2022. p. 19-44. 10.1079/9781789243505.0003
20. Peters. SO, Shoyebo, O. D., Ilori, B. M., MO, O., Adebambo, O. A., & Adebambo, O. Semen Quality Traits of Seven Strain of Chickens Raised in the Humid Tropics. *International Journal of Poultry Science*,2008: *7*(10), 949-953 DOI: 10.3923/ijps.2008.949.953
21. Chalah T, Brillard JP. Comparison of assessment of fowl sperm viability by eosin-nigrosin and dual fluorescence (SYBR-14/PI). Theriogenology. 1998;50(3):487-93. 10.1016/S0093-691X(98)00155-1
22. Fischer D, Schneider H, Failing K, Meinecke-Tillmann S, Wehrend A, Lierz M. Viability assessment of spermatozoa in large falcons (Falco spp.) using various staining protocols. Reprod Domest Anim. 2020;55(10):1383-92. 10.1111/rda.13785
23. Showell MG, Mackenzie-Proctor R, Brown J, Yazdani A, Stankiewicz MT, Hart RJ. Antioxidants for male subfertility. Cochrane Database Syst Rev. 2014;(12):CD007411. DOI: 10.1002/14651858.CD007411.pub3
24. Hernández-Avilés, C, Ramírez-Agámez, L, & Makloski-Cohorn, C. Semen evaluation. *Equine hematology, cytology, and clinical chemistry*, 2021; 257-274 10.1002/9781119500186.ch17
25. Ramu S, Jeyendran RS. The hypo-osmotic swelling test for evaluation of sperm membrane integrity. In: Spermatogenesis: Methods and protocols. Springer; 2012. p. 21-5. 10.1007/978-1-62703-038-0_3
26. Lelono, A, Riedstra, B & Groothuis, T. Ejaculate testosterone levels affect maternal investment in red junglefowl (Gallus gallus gallus). *Scientific Reports*, 2019; 9(1), 12126. 10.1038/s41598-019-48563-w
27. Isaac UE, Oyo-Ita E, Igwe NP, Ije EL. Preparation of histology slides and photomicrographs: Indispensable techniques in anatomic education. Anatomy Journal of Africa. 2023 Apr 6;12(1):2252-62. 10.4314/aja.v12i1.1
28. VerMilyea M, Hall JMM, Diakiw SM, Johnston A, Nguyen T, Perugini D, Miller A, Picou A, Murphy A P & Perugini, M. Development of an artificial intelligence-based assessment model for prediction of embryo viability using static images captured by optical light microscopy during IVF. Hum Reprod. 2020;35(4):770-84. 10.1093/humrep/deaa013
29. Holden SA, Fernandez-Fuertes B, Murphy C, Whelan H, O'gorman A, Brennan L, Butler ST, Lonergan P & Fair, S. Relationship between in vitro sperm functional assessments, seminal plasma composition, and field fertility after AI with either non-sorted or sex-sorted bull semen. Theriogenology. 2017; 87:221-8. 10.1016/j.theriogenology.2016.08.024
30. 29.Saito K, Kinoshita Y, Hosaka M. Direct and indirect effects of electrical stimulation on the motility of human sperm. Int J Urol. 1999;6(4):196-9. 10.1046/j.1442-2042.1999.06444.x
31. Chen YH, Yu JF, Chang YJ, Chin SC, Wang LC, Lin HL, & Tsai PS. Novel low-voltage electro-ejaculation approach for sperm collection from zoo captive Lanyu miniature pigs (Sus barbatus sumatranus). Animals. 2020;10(10):1825. 10.3390/ani10101825
32. Ansari, M. Recent strategies to mitigate reproductive aging in male broiler breeders: A review. *Animal Reproduction Science*, 2024, 268: 107570. 10.1016/j.anireprosci.2024.107570

تأثير تغيير الفولتية في تقنية القذف الكهربائي على جودة السائل المنوي، الكفاءة التناسلية، والنسيج الخصوي في الديوك الصغيرة والكبيرة في السن

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الخلاصة

هدفت هذه الدراسة إلى تقييم تأثير اختلاف جهد التحفيز الكهربائي على جودة السائل المنوي، الكفاءة التناسلية، ونسيج الخصية في ديوك صغيرة السن وكبيرة السن. أظهرت النتائج أن الديوك الكبيرة في السن حققت أفضل أداء عند استخدام جهد منخفض، بينما سجلت الديوك الصغيرة في السن أعلى معدلات الحركة الكلية، الحركة التقدمية، ومقاييس السرعة عند استخدام جهد متوسط. أدى استخدام جهد عالٍ إلى انخفاض معنوي في حركة الحيوانات المنوية في كلا المجموعتين، مع ملاحظة تأثير أكثر وضوحًا في الديوك الكبيرة في السن. بالنسبة لحجم السائل المنوي، تركيز الحيوانات المنوية، والحيوية، بلغت الديوك الصغيرة ذروتها عند الجهد المتوسط، بينما حققت الديوك الكبيرة أفضل النتائج عند الجهد المنخفض. كما تسببت المحفزات ذات الجهد العالي في تدهور جودة السائل المنوية وزيادة ملحوظة في تشوهات الحيوانات المنوية، خاصة في الديوك الكبيرة في السن. كما لوحظ أن مؤشر الغدد التناسلية في الديوك الصغيرة بلغ ذروته عند الجهد المتوسط ثم انخفض عند الجهود الأعلى، بينما لم تظهر الديوك الكبيرة تغيرات واضحة. كانت مستويات مؤشر الإجهاد التأكسدي(المالوندالديهايد) الأقل عند الجهد المتوسط في الديوك الصغيرة وعند الجهد المنخفض في الديوك الكبيرة. كما اتبعت سلامة غشاء البلازما وتركيزات التستوستيرون نفس النمط، حيث حققت الديوك الصغيرة أفضل النتائج التناسلية عند الجهد المتوسط، بينما سجلت الديوك الكبيرة أعلى القيم عند الجهد المنخفض. ومع ذلك، عانت كلا المجموعتين من تدهور معنوي في الخصوبة ومعدلات الفقس عند استخدام جهد عالٍ. كشف التحليل النسيجي أن الديوك الصغيرة في السن عند الجهد المتوسط احتفظت ببنية نيبيبات منوية جيدة، بينما أظهرت الديوك الكبيرة تغيرات تنكسيه مبكرة حتى عند الجهود المنخفضة والمتوسطة. أما عند الجهد العالي، فقد لوحظ تلف شديد في بنية الأنابيب المنوية وفقدان النطاف الناضجة في كلا المجموعتين. بشكل عام، تؤكد هذه النتائج على أهمية ضبط فولتية التحفيز الكهربائي وفقًا لعمر الديوك لتحسين جودة السائل المنوي والكفاءة التناسلية. يُوصى باستخدام فولتية متوسطة للديوك الصغيرة وفولتية منخفضة للديوك الكبيرة، بينما يجب تجنب الجهود العالية لما لها من آثار ضارة بغض النظر عن العمر.

33. Partyka A, Niżański W. Supplementation of avian semen extenders with antioxidants to improve semen quality-Is it an effective strategy? Antioxidants. 2021;10(12): 1927. 10.3390/antiox10121927
34. Partyka A, Łukaszewicz E, Niżański W. Lipid peroxidation and antioxidant enzymes activity in avian semen. Anim Reprod Sci. 2012;134(3-4):184-90. 10.1016/j.anireprosci.2012.07.007
35. Johnson SL, Dunleavy J, Gemmell NJ, Nakagawa S. Consistent age-dependent declines in human semen quality: a systematic review and meta-analysis. Ageing Res Rev. 2015; 19:22-33. 10.1016/j.arr.2014.10.007
36. Avital-Cohen, N., Heiblum, R., Argov-Argaman, N., Rosenstrauch, A., Chaiseha, Y., Mobarkey, N., & Rozenboim, I. Age-related changes in gonadal and serotonergic axes of broiler breeder roosters. Domestic Animal Endocrinology,2013, *44*(3), 145-150. 10.1016/j.domaniend.2013.01.002
37. Khayyat LI. The histopathological effects of an electromagnetic field on the kidney and testis of mice. Eurasia J Biosci. 2011; 5:103-9. 10.5053/ejobios.2011.5.0.12
38. Aydin M, Turk G, Yuksel M, Cevik A, Apaydin A, Yilmaz S. Effect of electromagnetic field on the sperm characteristics and histopathological status of testis in rats. Med Weter. 2007;63(2):178-83.
39. Sun Y, Xue F, Li Y, Fu L, Bai H, Ma H, Songshan X, Chen, J. Differences in semen quality, testicular histomorphology, fertility, reproductive hormone levels, and expression of candidate genes according to sperm motility in Beijing-You chickens. Poult Sci. 2019;98(9):4182-9. 10.3382/ps/pez208
40. Meel M, Jhamb D, Bansal KN, Gaur M, Meena AK, Jinagal S & Kumar P. Enhancing fertility and genetic progress in poultry through artificial insemination: current trends and innovations. Int J Bio-Resour Stress Manag. 2025;16(5). 10.23910/1.2025.6059
41. Ayeneshet B, Taye M, Esatu W, Tsefa A. Comparative analysis of semen quality and fertility in diverse rooster breeds: a systematic review. World's Poult Sci J. 2024;80(3):947-75. 10.1080/00439339.2024.2338347
42. Pintus E, Ros-Santaella JL. Impact of oxidative stress on male reproduction in domestic and wild animals. Antioxidants. 2021;10(7):1154. 10.3390/antiox10071154
43. Arif A, Zahoor N, Tang J, Tang M, Dong L, Khan S, Dai G. Cryopreservation strategies for poultry semen: A comprehensive review of techniques and applications. Veterinary Sciences, 2025; 12(2), p.145 10.3390/vetsci12020145
44. Khonmee J, Brown JL, Pérez AL, Lertwichaikul T, Sathanawongs A, Pornnimitra P, Areewong C,Supanta J, Punyapornwithaya V, Buddhasiri S, Punturee J. Effect of electroejaculation protocols on semen quality and concentrations of testosterone, cortisol, malondialdehyde, and creatine kinase in captive Bengal tigers. Animals. 2023;13(12):1893. 10.3390/ani13121893
45. Tabatabaei S, Chaji M, Mohammadabadi T. Correlation between age of rooster and semen quality in Iranian indigenous broiler breeder chickens. J Anim Vet Adv. 2010;9(1):195-8. 10.3923/javaa.2010.195.198