



## Original Article

# Relative Effectiveness of Management Techniques: Spiking and Intra-spiking on Broiler Breeder Production, Hatchability, and Fertility Traits



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## ABSTRACT

**Introduction:** The objective of the present study was to assess the impact of spiking and intra-spiking on mitigating the decline in fertility and hatchability associated with aging roosters in broiler breeder flocks.

**Materials & Methods:** A total of 162 Ross 308 strain birds were utilized, divided into six replicates, each consisting of nine individuals (eight hens and one cock). These replicates were randomly assigned to one of three management treatments: Group 1 (control group), group 2 (spiking group), or group 3 (intra-spiking group). Data were systematically collected from 42 to 62 weeks of age. Semen samples were obtained four times during the experimental period and evaluated for semen characteristics. Additionally, blood samples were supplied for quantifying concentrations of insulin, creatinine, testosterone, and nitric oxide. Following the experimental phase, each rooster was weighed and subsequently slaughtered, wherein testicular tissues were harvested for histological analysis. Fertility and hatchability rates were computed based on the collected data.

**Results:** Statistical analysis revealed no significant differences in semen characteristics among the experimental groups. However, the spiking group exhibited a statistically significant increase in testicular weight compared to the control and intra-spiking groups ( $P \leq 0.05$ ). Furthermore, there were greater counts of spermatocytes, spermatids, and spermatozoa in the spiking group ( $P \leq 0.05$ ). Conversely, the control group demonstrated elevated numbers of Sertoli cells, increased seminiferous duct diameters, and greater thickness of the germinal epithelium

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compared to the treatment groups ( $P \leq 0.05$ ). The decline in fertility and hatchability rates associated with advancing age was less pronounced in the spiking group when compared to the other experimental groups. While concentrations of insulin and creatinine did not exhibit significant variations among the groups, testosterone and nitric oxide levels were significantly higher in the spiking group ( $P \leq 0.05$ ).

**Conclusion:** The research findings indicate that spiking represents an effective strategy for maintaining fertility and enhancing hatchability rates in aged broiler flocks, as well as consequently increasing the economic profitability for broiler breeder producers.

## 1. Introduction

The natural decline in fertility among broiler breeder flocks with age poses a significant threat to economic production [1]. Fertility relies on the birds' productive status, interest, and mating capability [2]. As male birds age, mating activity decreases due to higher body weight, lower testosterone levels, and musculoskeletal diseases [3]. Fertility typically rises from 23-24 weeks, peaking at 35-37 weeks, and declines after 40-45 weeks, with older birds showing a more significant reduction [4]. To address this decline, various methods, particularly movement within rooster flocks such as intra-spiking and spiking, are employed.

Many industries employ spiking and intra-spiking, especially in older broiler breeder flocks. These practices involve introducing young, unfamiliar males or exchanging experienced males between poultry houses on the same farm. The goal is to enhance fertility levels in established flocks [5]. After reaching 40 weeks of age, breeder hens require more frequent mating to maintain fertility, while rooster mating interest typically declines [1]. Additionally, egg hatchability decreases with advancing age [6]. To address the fertility decline associated with aging roosters, various strategies focus on movement within rooster flocks, such as intra-spiking and spiking.

The male broiler breeder is crucial for ensuring egg fertility, often more so than the female [7]. Mating activity is influenced by male fitness and female receptivity, both of which can be affected by the sex ratio and feeding practices [8]. Introducing intra-spiking has shown potential to mitigate libido decline in aging males, thereby enhancing long-term flock fertility [1, 8].

Research indicates that incorporating 24-week-old males into a 45-week-old broiler breeder flock significantly increases hatchability, likely due to the heightened

libido of younger roosters. Additionally, females may preferentially mate with younger males, exhibiting pronounced secondary sex characteristics [9].

Intra-spiking involves relocating roosters within the same farm, allowing for the replacement of male broiler breeders without introducing external males [10]. This practice offers several advantages, including cost-effectiveness, reduced risk of disease transmission, ease of implementation, and rapid results [11]. However, while it may enhance mating activity, fertility can decline after 4-8 weeks due to the similar ages of the males. Typically, a fertility increase of 1-3% expected within 5-10 weeks post-spiking [10]. In contrast, a study by Patil and colleagues on the intra-spiking of Vanaraja chickens at 48 weeks revealed only a slight and statistically insignificant increase in fertility rates at 52 and 55 weeks, with no observed impact on hatchability. The effectiveness of spiking may vary depending on factors such as the age of the breeder flock, spiking frequency, and the ratio of exchanged roosters [4].

Male contribution to flock fertility is influenced by mating activity and sperm quality, both of which decline with age, leading to reduced semen volume and diminished fertilizing capability of spermatozoa [12].

To the authors' best knowledge, there has yet to be a comprehensive investigation into the effects of spiking and intra-spiking on sperm characteristics, testicular histology, sperm parameters, and various production factors. Therefore, the present study aims to evaluate the effectiveness of male spiking and intra-spiking on reproductive performance in Ross 308 broiler breeder flocks, with a focus on fertility, hatchability, testicular histology and sperm characteristics.

## 2. Materials and Methods

### 2.1. Birds, housing, and management

The research comprised three experiments involving 162 birds across three treatments, each with six replicates. Each replicate consisted of nine birds (8 hens and 1 cock). The birds were randomly assigned to one of following management treatments: Group 1 (control group, no management), group 2 (spiking group), and group 3 (intra-spiking group). The testing phase began when the birds reached 38 weeks of age, and data on experimental treatments were collected for weeks 40 to 62 (post-peak). Environmental conditions at the breeding farm, including moisture content, temperature, weight control, feed intake management, and other parameters, were maintained in accordance with the Ross 308 broiler breeder guide [13]. The experimental treatments included three management approaches: Standard recommended practices for Ross 308 broiler breeders, the spiking method (introducing a 28-week-old rooster to the flock), and the intra-spiking method (using roosters of the same age within halls or pens to stimulate early rooster mating activity). The ration composition, based on the Ross 308 nutrition guide for broiler chicken [14], included corn, wheat, soybean meal, oyster powder, calcium phosphate disodium, salt, mineral, and vitamin supplements, as well as methionine and lysine (Table 1).

### 2.2. Collection and evaluation of semen samples

Before the experiment began, semen was collected from each rooster at least three times to train the male birds [15]. Throughout the experiment, semen was collected four times and transferred to the laboratory in a warm water bath. Using an optical microscope, both qualitative and quantitative sperm characteristics were assessed, including semen volume, sperm density, sperm motility percentage, progressive sperm motility, and the percentage of dead and live sperm. Semen samples were collected using the abdominal rubbing method, which involved stimulating the roosters by rubbing their belly and back on the technician's leg to facilitate ejaculation. The ejaculated semen was collected using 1.5 mL graduated micro tubes. To prevent cold shock to the sperm, the micro tubes were carefully placed inside nylon bags before being transferred to a 37 °C warm water flask and transported to the laboratory. Upon arrival at the laboratory, the semen volume was promptly measured using the graduated markings body of the microtube. For the sperm motility assessment, samples were incubated at 37 °C for 30 minutes, then diluted in physiologic serum at a ratio of 1:200 mL. A drop of diluted sperm was then

examined under a microscope at 400x magnification, and progressive, non-progressive, and immotile sperm were conducted using a graded screen. Each specimen was evaluated for 200 to 400 sperm. Sperm concentration was determined by diluting the sample at a ratio of 1:400 mL in distilled water. A drop of the diluted sample was placed on a Neubauer chamber or Hemocytometer and examined under a microscope at 400x magnification. Semen concentration was calculated based on the count obtained from four lateral squares and one central square, using a Equation 1.

$$1. C = \frac{(NOSC \times D)}{5(NOS) \times 4(SCS)}$$

Where: C = concentration (nL), NOSC = count of sperm count, D = dilution, NOS = number of sperm counted per square, and SCS = sperm concentration in each home (nL).

To determine the proportion of live and dead spermatozoa in semen samples, a technique was employed involving the application of 10 µL of diluted specimen in distilled water (1:400 mL) onto a glass slide, followed by the addition of a single drop of Eosin-Nigrosine. Subsequently, a drop of the resultant solution was then spread at 45° on another glass slide. Following desiccation, a microscope magnified 400 was used to enumerate discolored spermatozoa (indicative of dead sperm) and those lacking color (representing viable sperm).

### 2.3. Determination of testicular characteristics

On day 156 of the experiment, testicular characteristics was conducted were assessed by randomly selecting and slaughtering a rooster from each nest. Before slaughtering, the roosters were weighed, followed by weighing of the testicles. Transverse sections with a maximum thickness of 0.5 cm were carefully prepared from each testicle. The tissue specimens were preserved in 10% formalin solution, and slides were prepared using established protocols. Quantification of spermatogonia, primary spermatocytes, spermatids, spermatozoa, Sertoli, and Leydig cells was conducted using a 10×10 grid scale graticule. Additionally, the seminiferous duct diameter of and germinal epithelial thickness were measured using a linear graticule.

### 2.4. Blood hormones and metabolites

Throughout the experiment, blood samples were systematically collected from roosters at 30-day intervals to assess levels of creatinine, insulin, testosterone, and

nitric oxide. Blood samples were drawn from the wing vein and centrifuged at 300 rpm for 20 minutes at a 4 °C. The separated serum was stored at a temperature of -20 °C until biochemical analysis. Subsequently, the samples were sent to the biochemical laboratory for detailed analysis.

#### 2.4. Calculation of fertility and hatchability

Fertility rate, hatchability rate, and hatch of fertility (HF) in chicken production in response to the experimental treatments was calculated using the Equations 2, 3 and 4:

2. Fertility rate (%) = (Total number of fertile eggs/Total number of eggs set)×100

3. Hatchability rate (%)=(Total number of chicks hatched / Total number of fertile eggs set)×100

4. HF=(Number of hatched chickens/(number of eggs-unfertilized or infertile eggs))×100

#### 2.5. Statistical data analysis

A balanced completely randomized design (CRD) and the MIXED model, which uses repeated measurements over time, were used for data analysis in SAS software, version 9.1. The statistical model incorporated the management method, sampling time, interaction between sampling time and management method, and random effects of roosters within each management method. The model structure followed an autoregressive pattern. For data without repetitive patterns over time (testicle weight and histological data), the general linear method (GLM) procedure was used. The least squares means (LSMeans) with standard error of means (SEM) were used to present the means in tables. To compare the means, Duncan's multiple-range test was used. A significance level of  $P \leq 0.05$  was considered statistically significant, while trends were discussed at  $0.05 \leq P \leq 0.10$ .

### 3. Results

#### 3.1. Qualitative and quantitative of semen characteristics

The effects of management techniques, spiking and intra-spiking, on semen parameters including volume (mL), concentration ( $\times 10^6$ ), motility, progress motility (%), and spermatozoa viability are depicted in Figure 1. As illustrated, no statistically significant differences were observed among the experimental groups.

#### 3.2. Testicular characteristics

The results of spiking and intra-spiking management methods on total testicles weights, as well as right and left testicular weights, are presented in Table 2. No significant differences in body weight were found between the experimental groups. However, both right and left testicles weights were significantly higher in the spiking group compared to the intra-spiking group ( $P \leq 0.05$ ).

Table 3 summarizes the histological data of the testicles in response to treatment. Treatments did not significantly affect the numbers of spermatogonia and Leydig cells. In contrast, the spiking management method increased the numbers of spermatocytes, spermatids, and spermatozoa cells compared to the other experimental groups ( $P \leq 0.05$ ). The number of Sertoli cells, seminiferous duct diameter, and germinal epithelium thickness were greater in the control group than in the two treatment groups ( $P \leq 0.05$ ).

#### 3.3. Fertility and hatchability

Percentage fertility, hatchability rate, and HF are presented in Tables 4 and 5. As shown in Table 4, there was no significant effect of management methods on the fertility of total eggs; however, the effect of time on total egg fertility was significant ( $P \leq 0.05$ ). A similar trend was observed in the infertility of total eggs. Table 4 also indicates that the percentage of infertile eggs increased with flock age, especially in the control group compared to the other two experimental groups. Although the reduction rate in the spiking group is lower than in the other two groups, the difference was not statistically significant.

In Table 5, the percentage of hatchability and hatchability of fertile eggs were not significantly affected by management methods. However, the effect of time was significant for both parameters ( $P \leq 0.05$ ).

#### 3.4. Blood hormones and metabolites

Figure 2 illustrates the analysis of blood parameters in response to management treatment.

Management treatments had no significant effect on blood creatinine and insulin concentrations. Testosterone concentration was affected by treatment during the first and second sampling times. In the first sampling time, the control group exhibited higher testosterone concentration than the other two experimental groups ( $P \leq 0.05$ ). In the second sampling time, the spiking group showed higher testosterone concentration than the other experimental groups ( $P \leq 0.05$ ).

**Table 1.** Nutrition and the dietary compound of broiler chicken ration

Components of Ration	%
Corn	53.44
Wheat	20.85
Oil	0.08
Soybean Meal	15.93
Oysters	7.45
Di-calcium phosphate	1.26
Salt	0.31
Vitamin supplement	0.25
Mineral supplement	0.25
DL-methionine	0.14
L-lysine hydrochloride	0.02
L-threonine	0.02
Metabolism energy (kcal/kg)	2.85
Protein (%)	14.44
Fat (%)	2.17
Linolenic acid (%)	1.4
Fiber (%)	3.84
Calcium (%)	3.3
P available (%)	0.34
Sodium (%)	0.22
Digestible arginine (%)	0.64
Digestible threonine (%)	0.46
Digestible lysine (%)	0.64
Digestible methionine (%)	0.35

Note: Vitamin and mineral supplements (in kg): Vitamin A: 4.4 g; vitamin D3: 0.72 g; vitamin B1: 0.306 g; vitamin B2: 1.5 g; vitamin B6: 0.306 g; vitamin B12: 1 g; vitamin E: 7.2 g; biotin: 1 g; vitamin K: 1 g; niacin: 2.48 g; folic acid: 0.306 g; pantothenic acid: 6.08 g; choline chloride: 220 g; manganese: 2 g; iron: 10 g; zinc: 13 g; iodine: 0.2 g; cobalt: 0.02 g; selenium: 0.04 g.

Regarding nitric oxide concentration during the experiment, unlike testosterone, significant difference was observed during the last three sampling times. Specifically, in the third, fourth, and fifth sampling times, the spiking group had a higher nitric oxide concentrations compared to the other experimental groups ( $P \leq 0.05$ ).

#### 4. Discussion

The reproductive potential of roosters is significantly influenced by both the quantity and quality of sperm produced in their testicles. In broiler breeder flocks, one rooster typically mates with multiple hens, emphasizing the importance of sperm characteristics in determining overall fertility flock. Three primary parameters are com-



**Table 2.** The average weights of the body and testicles between experimental groups at the end of the experiment with different management methods

Treatment	Body Weight (g)	Weight Testicles (g)	The Weight of the Right Testicle (g)	The Weight of the Left Testicle (g)
Control (no management)	5426.7	22.9 <sup>ab</sup>	11.5 <sup>ab</sup>	11.47 <sup>ab</sup>
Spiking	5940	34.83 <sup>a</sup>	18.83 <sup>a</sup>	16.33 <sup>a</sup>
Intra-spiking	5260	12.1 <sup>b</sup>	6.23 <sup>b</sup>	5.9 <sup>b</sup>
P	0.53	0.09	0.07	0.12
SEM	419.06	6.04	3.05	3.02

<sup>a, b, ab</sup>Significant differences between experimental groups ( $P \leq 0.05$ ).

monly utilized to assess sperm fertility: Concentration, viability, and mobility. As roosters age, reproductive performance declines, evidenced by reduced semen volume and a lower total number of spermatozoa per ejaculation. Additionally, older roosters may exhibit diminished fertilization capacity of their sperm cells, negatively impacting the fertility rates of the hens they mate with [16, 17]. In the present study, spiking and intra-spiking management techniques did not significantly affect the characteristics of sperm such as semen volume, sperm concentration, motility, progressive motility, or percentages of alive and dead sperm.

Male broiler chickens with body weights below 3,800 g are often infertility or subclinically infertile, typically associated with diminished testosterone levels and elevated corticosterone concentrations. Conversely, heavier male broilers tend to have larger and healthier testicular structures, alongside increased testosterone concentrations, and reduced corticosterone levels. Despite these advantageous physiological traits, heavier roosters en-

counter significant difficulties in mating due to their size. Factors such as heterogeneity among the male population, the establishment of a pecking order within the flock, and diminished hatch rates hinder effective mating and hen access.

Reproductive performance in roosters declines after 45 weeks, correlating with reductions in testicular weight, sperm production, and testosterone levels [16]. In the present study, the spiking group exhibited numerically higher body weight, this difference was not statistically significant. Notably, testicular size was significantly greater in the spiking group compared to the other two groups. The findings align with Frago et al., who reported that increased testicular size is closely linked to sperm production, as a considerable portion of testicular tissue is devoted to spermatogenesis [16]. Furthermore, existing research has demonstrated a positive correlation between age and testicular size, indicating that older roosters tend to possess larger testicles than their younger counterparts [18]. Increased testicular size, along with

**Table 3.** Average histological measurements of testicles in experimental groups at the end of the experiment period

Treatments	No.						Seminiferous Duct Diameter (μm)	Germinal Epithelium Thickness (μm)
	Spermato-gonia Cell	Spermato-cyte Cell	Spermatid Cell	Spermato-zoa Cell	Sertoli Cell	Leydig Cell		
Control (no management)	84.07	84 <sup>b</sup>	84.67 <sup>a</sup>	84.83 <sup>a</sup>	4.57 <sup>a</sup>	3.3	168.47 <sup>a</sup>	71.6 <sup>a</sup>
Spiking	87.63	94.4 <sup>a</sup>	93.6 <sup>a</sup>	90.23 <sup>a</sup>	3.33 <sup>b</sup>	2.9	149.87 <sup>b</sup>	52.67 <sup>b</sup>
Intra-spiking	85.07	56.13 <sup>c</sup>	53.57 <sup>b</sup>	37.33 <sup>b</sup>	3.9 <sup>ab</sup>	3	114.87 <sup>c</sup>	39.87 <sup>c</sup>
P	0.41	<0.01	<0.01	<0.01	0.005	0.32	<0.01	<0.01
SEM	1.95	3.47	4.12	5.39	0.26	0.19	4.54	1.59

Note: Different letters indicate significant differences between experimental groups ( $P \leq 0.05$ ).

**Table 4.** Main effects of management methods, spiking and intra-spiking, on the fertility of total eggs and infertility of total eggs (%) between experimental groups

Time	Fertility of Total Eggs				Infertility of Total Eggs			
	Treatments			Means of Time Effect	Treatments			Means of Time Effect
	1	2	3		1	2	3	
1	80	95.56	92.22	89.26 <sup>a</sup>	20	4.44	7.78	10.74 <sup>c</sup>
2	67.78	98.89	88.89	85.18 <sup>a</sup>	32.22	1.11	11.11	14.81 <sup>c</sup>
3	67.78	95.56	90	84.44 <sup>a</sup>	32.22	4.44	10	15.56 <sup>cb</sup>
4	77.78	94.44	78.89	83.7 <sup>a</sup>	22.22	5.56	21.11	16.3 <sup>cb</sup>
5	78.89	90	83.33	84.07 <sup>a</sup>	21.11	10	16.67	15.93 <sup>c</sup>
6	58.89	80	71.11	70 <sup>b</sup>	41.11	20	28.89	30 <sup>b</sup>
7	55.56	54.44	47.78	52.59 <sup>c</sup>	44.45	45.56	52.22	47.41 <sup>a</sup>
8	36.67	64.44	42.22	47.78 <sup>c</sup>	63.33	35.56	57.77	52.22 <sup>a</sup>
Means of treatments	65.42	84.17	74.31	Means of treatments	34.58	15.83	25.69	
	Treatment	Time	Treatment×time		Treatment	Time	Treatment×time	
P	0.21	<0.01	0.19		P	0.21	<0.01	0.19
SEM	7.16	5.82	10.07		SEM	7.17	5.82	10.07

<sup>a, b, c, cb</sup>Significant differences between experimental groups ( $P \leq 0.05$ ).

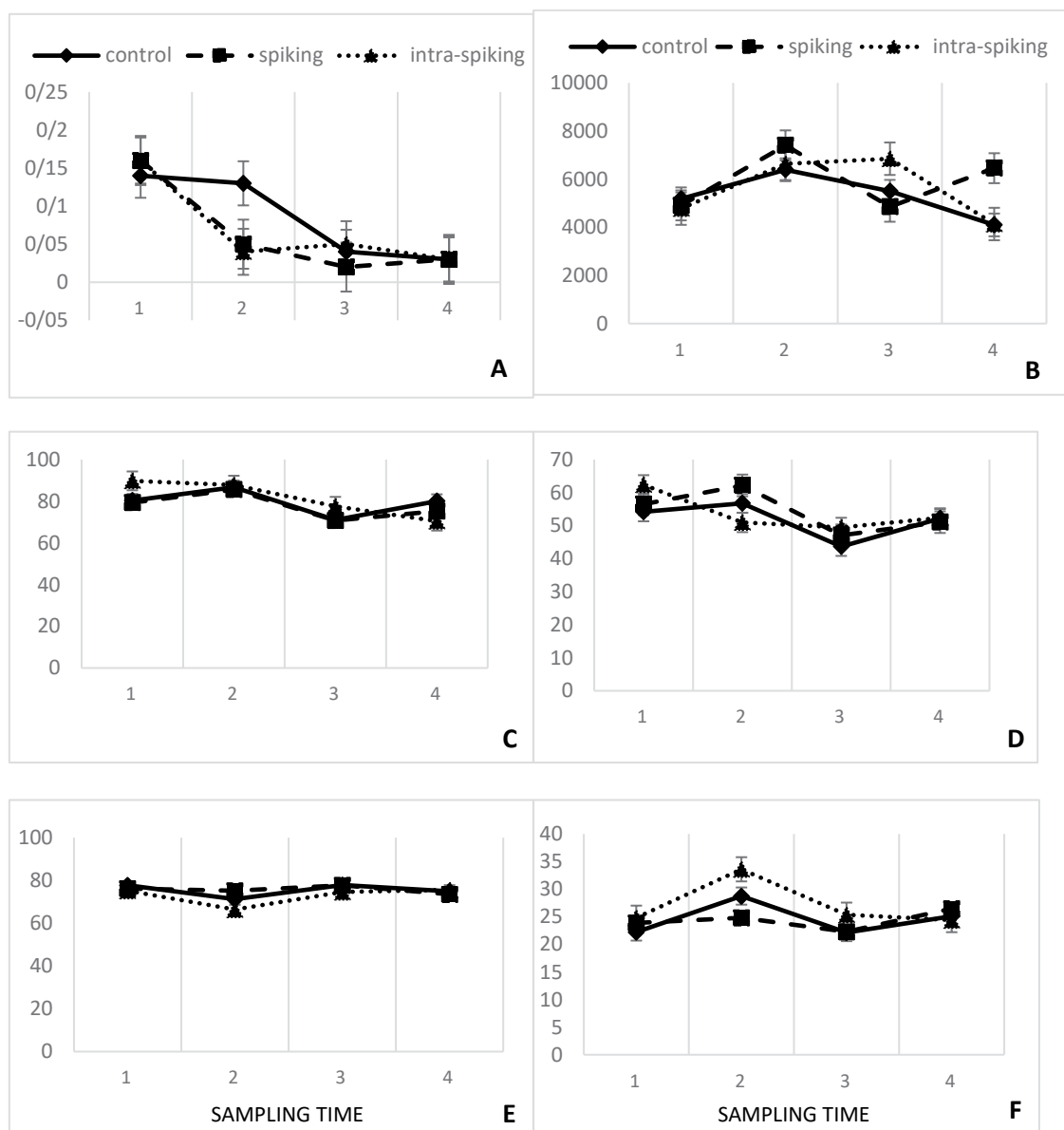
Note: Treatments include: 1: Control; 2: Spiking; 3: Intra-spiking.

a well- developed network of blood vessels supplying sufficient blood to the testicular tissue, is crucial for optimal spermatogenesis. However, when sperm are not subjected to a suitable environment, their lifespan is diminished both in the seminiferous ducts and the oviduct of chickens [19]. Additionally, aging leads to a noticeable decline in testicular quality, resulting in decreased sperm production and testosterone levels [16]. Fertility rates and the number of hatched chicks are essential metrics for evaluating successful reproduction, reflecting the overall efficacy of the mother flock. Infertility may indicate underlying issues within the male population. Globally, management techniques such as spiking and intra-spiking are frequently employed to mitigate reduced libido in aging roosters [1]. In the present study, the spiking method was implemented to reduce infertility rates and enhance overall egg fertility.

However, these changes did not yield statistically significant differences among the experimental groups. Similar patterns were observed in the hatchability of both total and fertile eggs. These findings contrast with those reported by other researchers [8, 20], who noted a

significant impact of the spiking method on egg fertility and hatchability, effectively reversing declines in these parameters. Conversely, some studies have indicated that spiking did not significantly affect egg fertility and hatchability [21, 22]. Fertility is directly linked to the characteristics of the parent flock. As the breeder flock ages, both egg fertility and hatchability tend to decrease, often as a result of reduced mating frequency, lower sperm motility, and decreased sperm penetration into the egg in aging broiler breeders [17].

Blood creatinine and insulin concentrations did not demonstrate statistically significant differences. However, testosterone levels exhibited a declining trend with increasing age of the roosters throughout the experimental period, regardless of treatment conditions. Specifically, the spiking group displayed significantly higher testosterone concentrations between 40 and 50 weeks of age compared to the other groups. Additionally, a notable distinction in nitric oxide concentration was observed in the spiking group between 50 and 60 weeks of age relative to the other groups. Testosterone is a critical hormone influencing age-related changes in



**Figure 1.** Changes in the volume (mL, A), concentration ( $\times 10^6$ , B), motility (%), progressive motility (%), live (%), and dead (%), of sperm between roosters in three experimental groups

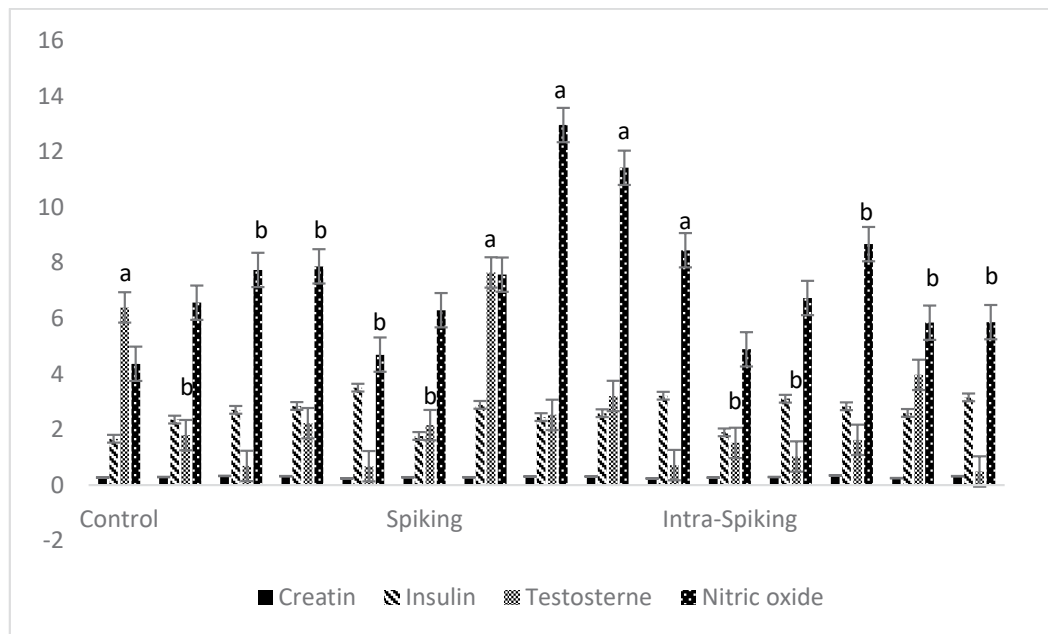
Note: Significance was considered at the level of 5% ( $P \leq 0.05$ ).

fertility, affecting various physiological traits such as testicular development, modulation of Sertoli cells, as well as sexual behavior, and mating activity [20]. The finding of this study align with previous research has established a positive correlation between sexual behavior and testosterone levels in roosters [4]. Furthermore, older male birds exhibit reduced testicular responsiveness to luteinizing hormone (LH) concerning testosterone production compared to their younger counterparts [23].

Nitric oxide, functioning as both an intra- and intercellular signaling molecule, plays a pivotal role in regulat-

ing hormone secretion. It exerts autocrine and paracrine biphasic control over steroidogenesis in Leydig cells [23]. In the present study, no significant differences in the quantity of Leydig cells were detected between the control and treatment groups. Nonetheless, further investigation is warranted to elucidate potential variations in Leydig cell activity among the experimental groups, highlighting the necessity for additional research to clarify this aspect.





**Figure 2.** Changes in the blood concentration of creatinine (mg/dL), insulin (μLU/mL), testosterone (ng/mL), and nitric oxide (μM) between cockerels in three managerial methods during the experiment. Sampling times: 40, 45, 50, 55, and 60 weeks of age

Note: Significance was considered at the level of 5% ( $P \leq 0.05$ ).

**Table 5.** Main effects of management methods, spiking and intra-spiking, on hatchability of total eggs and hatchability of fertile eggs (%) between experimental groups

Time	Hatchability of Total Eggs				Hatchability of Fertile Eggs			
	Treatments			Means of Time Effect	Treatments			Means of Time Effect
	1	2	3		1	2	3	
1	78.88	94.44	91.11	88.15 <sup>a</sup>	65.81	65.93	65.74	65.83 <sup>a</sup>
2	63.33	95.55	83.33	80.74 <sup>a</sup>	63.31	64.34	62.64	63.43 <sup>a</sup>
3	66.67	95.55	86.67	82.96 <sup>a</sup>	65.81	66.67	63.99	65.49 <sup>a</sup>
4	76.67	93.33	77.77	82.59 <sup>a</sup>	54.7	65.87	65.74	62.1 <sup>a</sup>
5	71.11	81.11	76.66	76.29 <sup>a</sup>	59.07	60.29	60.43	59.93 <sup>a</sup>
6	57.98	69.46	62.46	63.30 <sup>b</sup>	36.16	53.5	52.29	47.32 <sup>b</sup>
7	62.31	71.68	55.29	63.09 <sup>b</sup>	35.57	36.15	34.07	35.26 <sup>d</sup>
8	52.78	73.14	58.24	61.39 <sup>b</sup>	38.92	46.77	37.41	41.03 <sup>c</sup>
Means treatments	66.22	84.28	73.94		52.42	57.44	55.29	
	Treatment	Time	Treatment×time		Treatment	Time	Treatment×time	
P	0.18	0.002	0.64		P	0.14	<0.01	0.17
SEM	6.50	5.26	9.11		SEM	1.69	2.22	3.86

Note: Treatments include: 1: Control; 2: Spiking; 3: Intra-spiking. Different letters indicate significant differences between experimental groups ( $P \leq 0.05$ ).

## 5. Conclusion

The findings from the current study suggest that implementing management techniques, such as spiking, beginning at 40 weeks of age, may effectively mitigate the decline in fertility and hatchability rates observed in broiler breeder flocks. Timely application of management strategies is crucial as it addresses the physiological and reproductive challenges associated with aging in broiler breeders, which typically result in reduced reproductive performance. By utilizing spiking methods, it is possible to enhance the overall reproductive capacity of these flocks, ultimately leading to improved results in egg fertility and hatchability. The results underscore the importance of prompt intervention in management practices to maintain reproductive efficiency, particularly as the broiler breeder population ages. In conclusion, adopting these management strategies is essential for sustaining optimal reproductive performance within broiler breeder flocks. These approaches effectively counteract the premature decline in flock fertility, while also preserving the genetic potential of breeding birds. Prioritizing reproductive efficiency enables broiler breeder producers to achieve significant improvements in profitability while ensuring the sustainability of their breeding programs. Continued research into the long-term effects and best practices for these management techniques is necessary to develop comprehensive guidelines for practitioners in the field.

## Ethical Considerations

### Compliance with ethical guidelines

This study was approved by [Kowsar Agricultural Investment Company](#), Tehran, Iran, and [Animal Science Research Institute](#), Karaj, Iran.

As part of this experiment, all animal procedures and ethical considerations were performed following the Guide to the Care and Use of Agricultural Animals in Research and Teaching (FASS, 2010). Also, this study was conducted according to the procedures established by the Iranian [Ministry of Agriculture](#) (Experimental Authorization No. ASRI-2016-95014).

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## Authors' contributions

Data acquisition and analysis: All authors; Writing the original draft: Akbar Yaghobfar and Rezvan Yaghoubfar; Review and editing: Akbar Yaghobfar and Hoda Javaheri Barfourrooshi.

## Conflict of interest

The authors declared no conflict of interest.

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## References

- [1] Mphepya LC, van Rensburg WJ, Mpofu TJ, Mtileni BJ, Nephawe KA. Influence of male-male competition on reproductive performance and mortality of broiler breeders following intra-spiking. *Poult Sci.* 2019; 98(10):4549-54. [DOI:10.3382/ps/pez273] [PMID]
- [2] Volpe ALD, Slaviero A, Scher A, Molinari PV, Gerber MW, Marcon VM, et al. Effect of storage time, broiler breeder strain, and age on hatchability and first-week broiler performance. *Braz J Poult Sci.* 2024; 26(2):001-010. [DOI:10.1590/1806-9061-2024-1905]
- [3] Sabah S, Dikmen BY. Spiking applications in broiler breeders and its effect on reproduction performance. *World's Poult Sci J.* 2023; 79(3):581-91. [DOI:10.1080/00439339.2023.2208386]
- [4] Patil S, Khune VN, Mishra S, Singh N, Bobade MD, Bhagat V. Effect of interspiking on fertility and hatchability of vanaraja chicken parents. *J Anim Health Product.* 2019; 7(2):65-69. [DOI:10.17582/journal.jahp/2019/7.2.65.69]
- [5] Chung KM. Effect of double interspiking on fertility, behavior, and blood parameters in broiler breeder males reared under heat stress conditions [MA thesis]. Knoxville: University of Tennessee; 2010. [Link]
- [6] Peric L, Mitrovic M, Tomic B, Orehvacki I, Meijerhof R. Effects of flock age, place of oviposition, and cleaning treatments of hatching eggs on hatchability in broiler breeders. *J Appl Poult Res.* 2022; 31(3):100279. [DOI:10.1016/j.japr.2022.100279]
- [7] Wolc A, White IMS, Olori VE, Hill WG. Inheritance of fertility in broiler chickens. *Genet Sel Evol.* 2009; 41(1):47. [DOI:10.1186/1297-9686-41-47] [PMID]
- [8] Haghighi M, Irani M, Jafari M, Firouzi S, Habibi H. Effect of sex ratio on the production and hatchability of broiler breeder flock. *J World Poult Res.* 2016; 6(1):14-17. [Link]

- [9] Jafari M, Irani M, Haghighi M, Firouzi S, Babazadeh D. Effect of Spiking on Hatchability Levels and Testicular Weight in Broiler Breeder. *JWorld Poult Res.* 2015; 5 (2):29-31. [\[Link\]](#)
- [10] Sluis W. Spiking reduces fertility drop in older flocks. *Poult World.* 2014; 30(7):20-1. [\[Link\]](#)
- [11] Meijerhof R. Poultry Performance Plus: Intra Spiking [Internet]. 2022 [Updated 13 March 2022]. Available from: [\[Link\]](#)
- [12] Mangiagalli MG, Martino PA, Smajlovic T, Guidobono Cavalchini L, Marelli SP. Effect of lycopene on semen quality, fertility and native immunity of broiler breeder. *Br Poult Sci.* 2010; 51(1):152-7. [\[DOI:10.1080/00071660903401540\]](#) [\[PMID\]](#)
- [13] Aviagen. Parent Stock. Ross 308 Parent Stock: Performance objectives. In: Ross parent stock management. Huntsville: Ross an Aviagen; 2016. [\[Link\]](#)
- [14] Aviagen. Parent Stock Nutrition Specifications: Ross 308. Huntsville: Ross an Aviagen; 2016.
- [15] Castillo A, Lenzi C, Pirone A, Baglini A, Russo C, Soglia D, et al. From the Semen Collection Method to the Hatchlings: The Use of Cryopreserved Sperm from Pheasants Fed an Antioxidant-Enriched Diet. *Animals.* 2021; 11(9):2624. [\[DOI:10.3390/ani11092624\]](#) [\[PMID\]](#)
- [16] Fragoso JS, Pizarro Díaz M, Moreno JCA, Casanovas IP, Rodríguez-Bertos A, Barger, K. Relationships between fertility and some parameters in male broiler breeders (body and testicular weight, histology and immunohistochemistry of testes, spermatogenesis and hormonal levels). *Reprod Domest Anim.* 2013; 48(2):345-52. [\[DOI:10.1111/j.1439-0531.2012.02161.x\]](#) [\[PMID\]](#)
- [17] Liang W, He Y, Zhu T, Zhang B, Liu S, Guo H, et al. Dietary restriction promote sperm remodeling in aged roosters based on transcriptome analysis. *BMC Genomics.* 2024; 25(1):680. [\[DOI:10.1186/s12864-024-10544-3\]](#) [\[PMID\]](#)
- [18] Laskemoen T, Fossoy F, Rudolfsen G, Lifjeld JT. Age-related variation in primary sexual characters in a passerine with male age-related fertilization success, the Bluethroat, *Luscinia svecica*. *J Avian Biol.* 2008; 39(3):322-8. [\[DOI:10.1111/j.0908-8857.2008.04178.x\]](#)
- [19] Mfoundou JDL, Guo Y, Yan Z, Wang X. Morpho-Histology and Morphometry of Chicken Testes and Seminiferous Tubules among Yellow-Feathered Broilers of Different Ages. *Vet Sci.* 2022; 9(9):485. [\[DOI:10.3390/vetsci9090485\]](#) [\[PMID\]](#)
- [20] Ordas B, Vahedi S, Seidavi A, Rahati M, Laudadio V, Tufarelli V. Effect of testosterone administration and spiking on reproductive success of broiler breeder flocks. *Reprod Domest Anim.* 2015; 50(5):820-5. [\[DOI:10.1111/rda.12595\]](#) [\[PMID\]](#)
- [21] Nazarpak HH, Fallah-Khair AR. Effect of spiking of young males in different percentage on economic performance of broiler breeder flock. *J Food Agric Environ.* 2011; 9(3/4):564-9. [\[Link\]](#)
- [22] Chung KM, Smith MO, Kattesh HG. The influence of double interspiking on production and behavior in broiler breeder flocks in elevated temperature conditions. *J Appl Poult Res.* 2012; 21(1):63-9. [\[DOI:10.3382/japr.2011-00347\]](#)
- [23] Lengyel K, Rudra M, Berghof TVL, Leitão A, Frankl-Vilches C, Dittrich F, et al. Unveiling the critical role of androgen receptor signaling in avian sexual development. *Nat Commun.* 2024; 15(1):8970. [\[DOI:10.1038/s41467-024-52989-w\]](#) [\[PMID\]](#)

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