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Analysis of ejaculate parameters and sperm morphology in roosters of initial laying strains

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Abstract: The aim of this study was to evaluate the influence of laying hybrid lines of domestic fowl (*Gallus gallus f. domestica*) and their age on ejaculate parameters in roosters. Parameters assessed included ejaculate volume, sperm concentration, total sperm count (TSC), percentage of normal spermatozoa, and sperm motility. Additionally, morphological defects were observed: defects of the acrosome, head, neck, tail, and immature spermatozoa. The study included 120 roosters of the Barred Plymouth Rock, Sussex Light, Rhode Island Red, and Rhode Island White laying lines. Ejaculate was repeatedly evaluated at the ages of 34, 43, and 52 weeks. The average ejaculate volume was 0.52 ml, sperm concentration 2.53 × 10^9 cm^{-3}, motility 81.1%, and defect occurrence rates were as follows: acrosome defects 0.7%, head defects 3.0%, neck defects 2.4%, and tail defects 2.9%. A statistically significant effect ($P < 0.01$) of the line was observed for all parameters except tail defects. Statistically significant differences between age categories were confirmed for volume, sperm concentration, TSC, immature spermatozoa, and normal spermatozoa ($P < 0.01$), as well as for ejaculate volume, neck, and tail defects ($P < 0.05$). The volume, concentration and TSC reached significantly higher values at 43 weeks of age. The incidence of normal spermatozoa and immature spermatozoa was significantly lower at 34 weeks of age. The interaction of age and line was significant ($P < 0.01$) for sperm concentration, TSC, motility, head, tail, and normal spermatozoa. The highest values of volume, concentration, and TSC were observed in the BPR line, which also exhibited the lowest occurrence of head defects and immature spermatozoa. The RIR line showed a higher occurrence of sperm defects. Younger roosters (34 and 43 weeks of age) had higher ejaculate parameter values and a higher occurrence of sperm defects compared to the older ones.

Keywords: acrosome; abnormal spermatozoa; chicken; hybrid lines; motility

Evaluation of basic ejaculate parameters and morphological defects in initial laying strains of domestic fowl is essential for successful reproduction and, consequently, for the entire industry of laying hybrid production. Numerous authors have addressed the evaluation of sperm parameters (Meamar et al. 2016; Lukaszewicz et al. 2020; Jimoh et al. 2021; Shaheen et al. 2023; Ghadimi et al. 2024). The qual-

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ity of ejaculate, and thereby fertility, is influenced by a variety of factors. Among the significant factors is rooster nutrition, particularly the use of various feed additives, which have been documented to positively affect ejaculate quality. These additives include, for example, lutein (Li et al. 2023), selenium (Yan et al. 2024), zinc (Jafari et al. 2021), other minerals (Londero et al. 2020), and numerous other substances, including vitamins and herbal supplements.

Other non-genetic factors include the influence of housing conditions (Shaheen et al. 2023) and the frequency of ejaculate collection (Pimprasert et al. 2023; Shaheen et al. 2023). The impact of access to pasture on ejaculate quality was also studied in two native Mediterranean breeds (Santiago-Moreno et al. 2018). Another important factor is the age of roosters, which was also evaluated in broiler roosters (Shaheen et al. 2023). The authors of this study confirmed, alongside the effects of housing and collection frequency, a significant influence of age on ejaculate parameters. Roosters aged 25–45 weeks produced ejaculates with higher volume and sperm concentration compared to those aged 46–65 weeks. Another study on broiler roosters similarly confirmed a decline in ejaculate parameters, particularly in sperm concentration and volume, with age, as well as an increase in sperm defects in older roosters (Tabatabaei et al. 2010).

The influence of age and strain, as well as their interaction, was addressed in a study comparing the laying strain White Leghorn and the dual-purpose Beijing You Chicken (Shi et al. 2021). The authors highlighted a significant effect of strain on ejaculate volume and total sperm count, as well as an effect of age on ejaculate volume. Furthermore, a significant interaction between strain and age was observed in relation to ejaculate volume.

The comparison of ejaculate parameters in laying strains, specifically White Leghorn and Rhode Island Red roosters, was the focus of the study by Tesfay et al. (2020). Significant differences between strains were noted in ejaculate volume, pH, sperm motility, viability, and abnormal sperm percentage. The study emphasised the importance of evaluating these parameters in individual strains and the necessity of selection for ejaculate traits within these strains to improve fertility. This requires knowledge of the heritability of ejaculate parameters, as examined for White Leghorn by Wolc et al. (2019).

The occurrence of defects in spermatozoa of both broiler and layer roosters has been extensively studied (Tabatabaei et al. 2009; Masoudi et al. 2018; Rahnama et al. 2020; Heng et al. 2022). Abnormalities in broiler sperm were also investigated in the study by Tapeh et al. (2017). A comparison with other livestock species was presented by Egyptien et al. (2023). Furthermore, the analysis of ejaculate parameters and spermatozoa in layer strains in the Czech Republic was the subject of several studies (Machal and Krivanek 2002; Jarinkovicova et al. 2012).

From the aforementioned research, significant differences between age and strain have been observed for key ejaculate parameters, yet these studies lack in-depth analysis, particularly for morphological indicators of spermatozoa. Additionally, a considerable portion of research focuses solely on broiler roosters or specific local layer strains.

The objective of this study was to evaluate the effect of rooster strain and age on ejaculate volume, concentration, total sperm count, motility, and selected morphological abnormalities of sperm. The null hypothesis posited that the mean values of the studied ejaculate parameters and sperm morphological abnormalities do not differ across strains, age groups, or their interactions.

MATERIAL AND METHODS

The study was conducted according to the guidelines of the Declaration of Helsinki. Experimental procedures and animal care conditions followed the recommendation of European Union Directive 86/609/EEC and were approved by Expert Commission for Ensuring the Welfare of Experimental Animals of Mendel University in Brno. Ethic Committee Name: The Ethics Review Board (The Ethics Committee of Expert Commission for Ensuring the Welfare of Experimental Animals) of Mendel University in Brno. Approval Code: 16OZ27083/2014-17214. Approval Date: 20 May 2019.

Ejaculates were collected over a 20-week period from six layer lines of four breeds: Barred Plymouth Rock (BPR A, BPR B, BPR C lines), Rhode Island Red (RIR line), Rhode Island White (RIW line), and Sussex Light (SU line). These rooster lines are used in the production of final layer hybrids in the Czech Republic. The roosters were reared in litter-based

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housing under standard conditions specified by the breeding program. At 16 weeks of age, the roosters were transferred from rearing facilities to adult rooster cages.

In the cages, the roosters were housed individually and fed a complete feed mixture restricted to 120 g/day. They had continuous access to drinking water via nipple drinkers. The light regime during the trial was 12 h of light and 12 h of darkness.

The study included 120 roosters whose ejaculates were collected repeatedly at different ages: 34 weeks (Age1), 43 weeks (Age2), and 52 weeks (Age3). A total of 337 samples were analysed. The number of observations per line is detailed in Table 1, and the distribution by age category is in Table 2. The total number of observations excludes some ejaculates for which neither basic parameter evaluation nor defect assessment was possible, or data were removed as outliers.

The recorded values were grouped into three age categories and six line groups. Each rooster was individually identified to include the random effect of individual roosters in the calculations (repeated measures).

Ejaculates were collected using the dorso-abdominal massage method. Immediately after collection, the following parameters were assessed: ejaculate volume (ml), sperm motility (%), sperm concentration (10^9 cm^{-3}), total sperm count (TSC), and sperm morphology (%). Ejaculate volume was determined using a calibrated pipette. Sperm motility was assessed by subjective evaluation. For sperm

Table 1. Mean values of semen parameters in different laying strains

Trait	Strain (mean ± SEM)					
	BPR A (N = 55)	BPR B (N = 58)	BPR C (N = 60)	RIR (N = 55)	RIW (N = 56)	SU (N = 53)
Volume (ml)	0.64 ± 0.05^{a}	0.56 ± 0.04^{a}	0.35 ± 0.03^{b}	0.26 ± 0.02^{b}	0.61 ± 0.05^{a}	0.35 ± 0.03^{b}
Concentration (10^9 cm^{-3})	2.44 ± 0.15^{b}	3.44 ± 0.24^{a}	2.29 ± 0.16^{b}	1.92 ± 0.15^{b}	2.29 ± 0.15^{b}	2.23 ± 0.11^{b}
TCS (10^9)	1.80 ± 0.17^{a}	2.10 ± 0.18^{a}	1.02 ± 0.12^{bc}	0.70 ± 0.09^{b}	1.50 ± 0.13^{ac}	0.92 ± 0.10^{b}
Motility (%)	75.7 ± 2.6^{b}	79.8 ± 2.0^{b}	80.0 ± 1.8^{b}	88.0 ± 0.8^{a}	78.3 ± 1.9^{b}	76.5 ± 1.5^{b}
Head (%)	1.9 ± 0.2^{ac}	1.2 ± 0.2^{a}	2.9 ± 0.4^{bc}	4.6 ± 0.4^{b}	2.1 ± 0.3^{c}	4.6 ± 0.4^{b}
Acrosome (%)	0.6 ± 0.1^{ab}	0.2 ± 0.1^{c}	0.7 ± 0.2^{ab}	0.8 ± 0.2^{ab}	0.4 ± 0.1^{bc}	1.3 ± 0.2^{a}
Neck (%)	3.9 ± 0.4^{a}	4.5 ± 0.3^{a}	0.2 ± 0.1^{b}	0.1 ± 0.1^{b}	5.2 ± 0.7^{a}	0.0 ± 0.0^{c}
Tail (%)	3.0 ± 0.4^{a}	2.6 ± 0.3^{a}	2.2 ± 0.2^{a}	2.8 ± 0.3^{a}	3.5 ± 0.3^{a}	2.7 ± 0.4^{a}
IS (%)	0.1 ± 0.0^{a}	0.0 ± 0.0^{b}	1.1 ± 0.2^{c}	1.6 ± 0.2^{c}	0.1 ± 0.1^{a}	1.2 ± 0.3^{c}
NS (%)	89.5 ± 0.6^{b}	89.9 ± 0.5^{b}	92.4 ± 0.5^{a}	90.1 ± 0.7^{ab}	87.3 ± 0.9^{b}	90.2 ± 1.0^{ab}

IS = immature spermatozoa; N = number of observations; NS = normal spermatozoa; SEM = standard error of the mean; TSC = total sperm count

$^{a–c}$Different superscripts in lines denote statistically significant differences ($P < 0.05$)

Table 2. Descriptive statistics of the dataset

Trait	Mean	SD	Min	Max	Number of observations
Volume (ml)	0.52	0.26	0.05	1.42	337
Concentration (10^9 cm^{-3})	2.53	1.60	0.09	9.85	337
TCS (10^9)	1.41	1.23	0.02	7.91	337
Motility (%)	81.1	13.8	20.0	95.0	337
Head (%)	3.0	2.5	0.0	17.0	337
Acrosome (%)	0.7	1.0	0.0	6.0	337
Neck (%)	2.4	3.1	0.0	15.0	337
Tail (%)	2.9	2.3	0.0	14.5	337
Immature spermatozoa (%)	0.7	1.2	0.0	6.0	337
Normal spermatozoa (%)	89.6	4.8	70.0	98.0	337

SD = standard deviation; TSC = total sperm count

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concentration, the haemocytometric method was employed.

Immediately after collection, a sample of the ejaculate was fixed for morphological evaluation of the sperm using a fixative solution of formaldehyde and sodium citrate (1 ml of 10% formaldehyde and 99 ml of 2.5% sodium citrate) at a 1 : 5 ratio. From the prepared suspension, a slide was made and examined under a phase-contrast microscope. For each sample, 200 sperm cells were evaluated, and the following morphological changes were observed: changes in the head, changes in the acrosome, changes in the neck (midpiece), changes in the tail, immature spermatozoa, and morphologically normal spermatozoa. The frequencies of each morphological change were expressed as percentages.

Statistical analysis

The data were analysed using the R statistical software (R Development Core Team 2022). The influence of the line and age on selected ejaculate parameters was tested using the GLM method with a random effect of rooster (repeated, correlated measurements). The NLME package or GEEGLM was used in R. The significance of the effects of individual factors was assessed using the *F*-test or Chi-square test. Subsequent differences between classes were evaluated using the Tukey-Kramer test.

None of the dependent variables met the criteria for normal distribution of frequencies or homogeneity of variances (Shapiro-Wilk test of normality and Bartlett's test, respectively). For ejaculate volume, a logarithmic transformation was applied (*F* test). Concentration and total sperm count (TSC) were analysed using a linear model with a Poisson distribution of frequencies (Chi-square test). For motility and the occurrence of morphological sperm abnormalities, a GLM with a binomial distribution of frequencies (Chi-square test) was used, and the results were expressed as percentages. The null hypothesis was rejected in all cases when $P < 0.05$.

RESULTS

Table 2 presents the basic ejaculate parameters for the initial sample. The average ejaculate volume across all 337 observed collections from 120 roosters was 0.52 ml. The sperm concentration per millilitre averaged 2.53×10^9 cm^{-3}, and the total sperm count in the ejaculate was on average 1.41×10^9. Sperm motility averaged around 81.1%, and the occurrence of normal spermatozoa was approximately 90%. Among the morphological abnormalities, the highest average occurrence was observed in head defects (3.0%), with some collections reaching up to 17%. The least frequent abnormalities were acrosome defects and immature spermatozoa, with an average occurrence of less than 1%. In some collections, there was no occurrence of pathological sperm, including all observed morphological abnormalities. The minimum motility reached 20%, and the minimum percentage of normal spermatozoa was 70%.

Table 3. Effect of strain and age on semen parameters

Trait	Test, distribution	*P*-value		
		strain	age	interaction strain–age
Volume (ml)	*F*-test, log normal	< 0.001	0.043	0.156
Concentration (10^6 cm^{-3})	Chi square, poisson	< 0.001	< 0.001	< 0.001
TCS (10^6)	Chi square, poisson	< 0.001	< 0.001	< 0.001
Motility (%)	Chi square, binomial	< 0.001	0.116	< 0.001
Head (%)	Chi square, binomial	< 0.001	0.147	< 0.001
Acrosome (%)	Chi square, binomial	< 0.001	< 0.001	0.100
Neck (%)	Chi square, binomial	< 0.001	0.075	0.136
Tail (%)	Chi square, binomial	0.176	0.034	< 0.001
Immature spermatozoa (%)	Chi square, binomial	< 0.001	0.001	0.326
Normal spermatozoa (%)	Chi square, binomial	0.001	< 0.001	< 0.001

TSC = total sperm count

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The highest variability, expressed as the coefficient of variation, was observed in the occurrence of immature spermatozoa (157%), followed by acrosome defects (138%) and neck defects (133%). In contrast, the lowest variability was found in sperm motility (17%) and the occurrence of normal spermatozoa (5%). Other parameters exhibited variability ranging from 50% to 90%.

Table 3 presents the statistical significance of the effects of line, age, and their interaction on the various semen parameters. A statistically significant effect of the line was observed on all parameters, except for tail defects (P = 0.176 1). Age had a highly significant effect on sperm concentration, total sperm count (TSC), acrosome defects, and the occurrence of immature and normal spermatozoa. A statistically significant effect of the age was also found on ejaculate volume (P = 0.043) and tail defects (P = 0.034). However, the age did not significantly affect motility, head defects, or neck defects. As for the interaction between line and age, no significant effects were observed on ejaculate volume, acrosome defects, neck defects, or the occurrence of immature spermatozoa.

Table 1 presents the values of the observed parameters for each line. The highest ejaculate volume was found in the RIW, BPR A, and BPR B lines (0.56–0.64 ml), which was statistically significantly higher than in the BPR C, RIR, and SU lines (0.26–0.35 ml). The highest sperm concentration (3.44×10^9 cm^{-3}) was observed in the BPR B line, which was statistically significantly different from the concentrations measured in the other lines. No significant differences in sperm concentration were found between the other lines. The total sperm count (TSC) was significantly higher in the BPR A, BPR B, and RIW lines, which also had higher ejaculate volumes. The lowest TSC values were found in the RIR line. Conversely, the RIR line exhibited the highest motility percentage (88%), with no statistically significant differences in motility between the other lines.

The occurrence of individual defects shows significant variability between the lines, with the exception of tail defects, where the incidence ranges from 2.2% to 3.5%, but the values are not statistically significantly different. Acrosome defects were most commonly observed in the SU line (1.3%), which was statistically different from the lowest values observed in the RIW and BPR B lines (0.4% and 0.2%, respectively). Head defects were most frequently found in the RIR and SU lines (both at 4.6%), while the BPR B (1.2%) and BPR A (1.9%) lines had lower values.

The largest differences between lines were observed in neck defects, where no occurrence was recorded in the SU line, and only 0.1% was found in the RIR line. In contrast, the occurrence of neck defects was 5.2% in the RIW line. Immature spermatozoa were absent in the BPR B line, and the minimal occurrence was seen in the BPR A line (0.1%). In comparison, statistically significant differences in the occurrence of immature spermatozoa were observed in the BPR C, RIR, and SU lines, where the incidence was higher than 1%. The highest percentage of normal spermatozoa was found

Table 4. Mean values of semen parameters at different age of roosters

Trait	Age (mean ± SEM)		
	Age 1 (34 weeks, N = 103)	Age 2 (43 weeks, N = 115)	Age 3 (52 weeks, N = 119)
Volume (ml)	0.43 ± 0.02^{ab}	0.48 ± 0.02^{a}	0.41 ± 0.02^{b}
Concentration (10^9 cm^{-3})	2.17 ± 0.09^{b}	3.06 ± 0.17^{a}	2.07 ± 0.09^{b}
TCS (10^9)	1.14 ± 0.08^{b}	1.73 ± 0.15^{a}	0.97 ± 0.06^{b}
Motility (%)	77.8 ± 1.4^{a}	80.0 ± 1.3^{a}	82.3 ± 1.1^{a}
Head (%)	2.8 ± 0.2^{a}	2.6 ± 0.2^{a}	2.4 ± 0.2^{a}
Acrosome (%)	0.8 ± 0.1^{a}	0.6 ± 0.1^{a}	0.4 ± 0.1^{b}
Neck (%)	2.2 ± 0.4^{a}	2.4 ± 0.3^{a}	2.5 ± 0.4^{a}
Tail (%)	3.4 ± 0.3^{a}	2.8 ± 0.2^{ab}	2.2 ± 0.2^{b}
Immature spermatozoa (%)	0.0 ± 0.0^{a}	0.4 ± 0.3^{b}	0.3 ± 0.2^{b}
Normal spermatozoa (%)	88.3 ± 0.5^{a}	90.5 ± 0.3^{b}	91.1 ± 0.5^{b}

N = number of observations; SEM = standard error of the mean; TSC = total sperm count

^{a,b}Different superscripts in lines denote statistically significant differences (P < 0.05)

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in the BPR C line (92.4%), which was statistically different from the number of normal spermatozoa observed in the BPR A, BPR B, and RIW lines (87.3–89.9%).

Table 4 presents the values of the monitored parameters depending on the rooster age. The ejaculate volume of roosters was statistically significantly different in the oldest age category (52 weeks), which had an average ejaculate volume of 0.41 ml, compared to the other two age categories (Age 1 = 34 weeks and Age 2 = 43 weeks), which had ejaculate volumes of 0.43 ml and 0.48 ml, respectively. The highest sperm concentration was achieved in the Age 2 category (3.06×10^9 cm^{-3}), with no statistically significant differences in sperm concentration between the youngest and oldest age categories. A similar trend was observed for the total sperm count (TSC).

In terms of sperm motility, no statistically significant differences were observed between the age categories, and the same applies to the incidence of head and neck defects. The occurrence of acrosome defects in the oldest age category (52 weeks) was statistically different from the two younger age categories, with a value of 0.4% compared to 0.80% and 0.60%, respectively. There was also a noticeable trend for the tail defect incidence to be lower in the older age category, with the youngest age category (Age 1) showing a statistically higher incidence of tail defects (3.4%), while the oldest age category (Age 3) had only 2.2%. Immature spermatozoa were not present in the youngest age category, and in the remaining age categories, their occurrence ranged from 0.3% to 0.4%. The occurrence of normal spermatozoa was statistically significantly different in the youngest age category (Age 1), where the ejaculate contained an average of 88.3% normal spermatozoa. In contrast, the incidence of normal spermatozoa was higher than 90% in the other two age categories (Age 2 and Age 3).

In Figures 1 to 6, the interaction between the line and age of the roosters is depicted for parameters where the interaction effect was found to be statistically significant (as shown in Table 3). In Figure 1, the interaction for sperm concentration is shown. For the BPR C, SU, and RIR lines, a noticeable increase in sperm concentration is observed in the middle age category (43 weeks), whereas for the other lines,

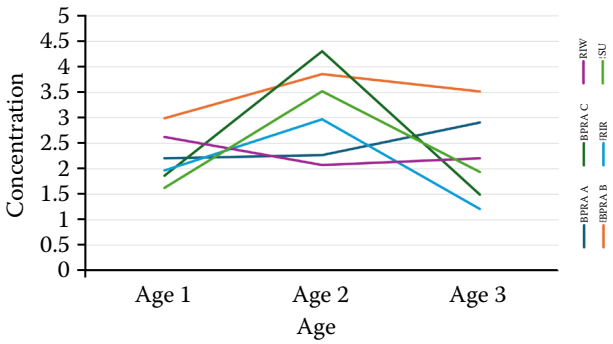

Figure 1. Effect of strain and age interaction on sperm concentration

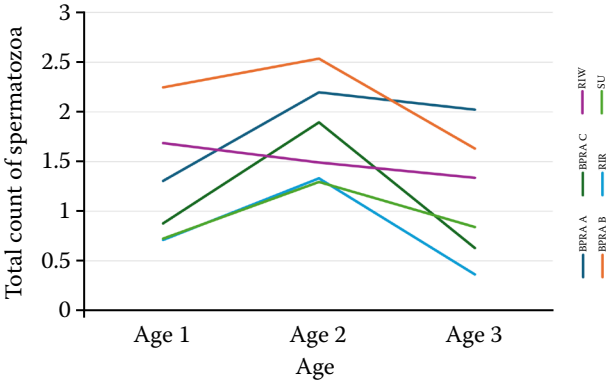

Figure 2. Effect of strain and age interaction on total sperm count

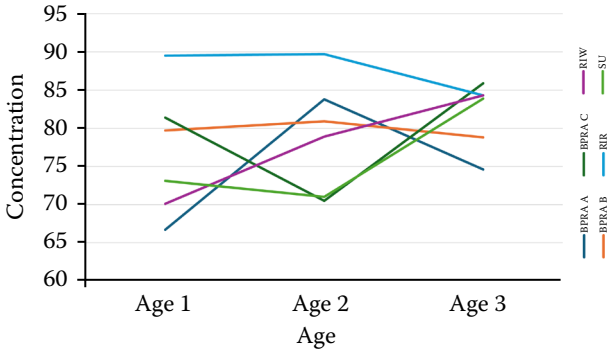

Figure 3. Effect of strain and age interaction on sperm motility

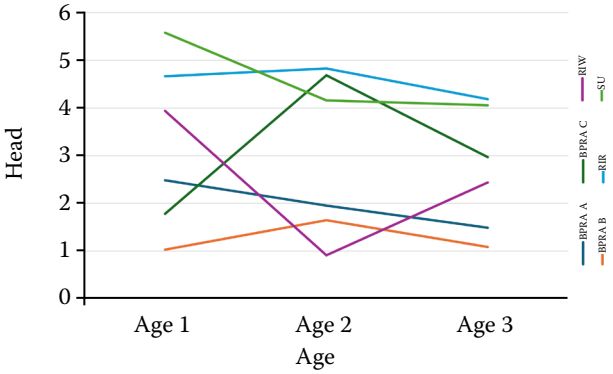

Figure 4. Effect of strain and age interaction on the occurrence of head defects

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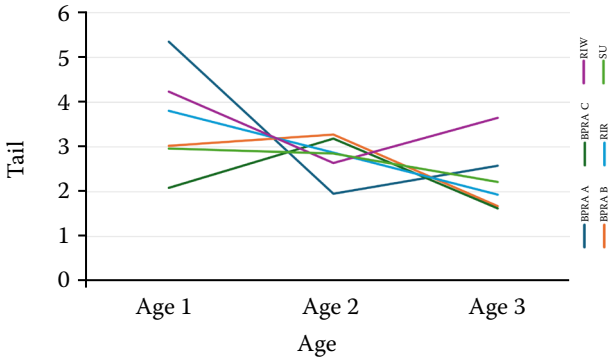

Figure 5. Effect of strain and age interaction on the occurrence of tail defects

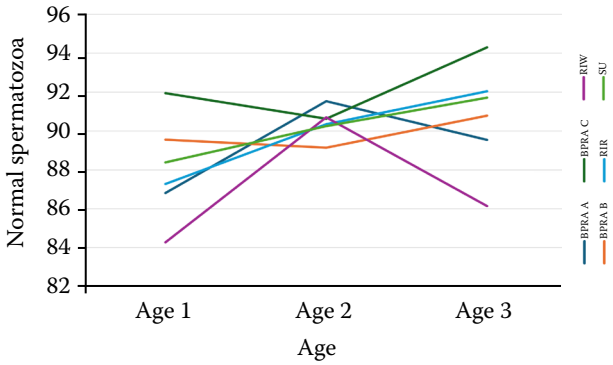

Figure 6. Effect of strain and age interaction on the occurrence of normal spermatozoa

there is no apparent difference in sperm concentration across the different age categories.

Figure 2 describes the interaction for TSC. A difference in TSC across the age categories is noticeable for some lines. Particularly, the RIW line shows a different trend of TSC compared to the other lines, with no increase in TSC from age category 1 (34 weeks) to age category 2 (43 weeks). The BPR C line, on the other hand, shows a significant decrease in TSC between age categories 2 and 3 (52 weeks), whereas in the other lines, this decrease is not statistically significant.

Figure 3 represents the interaction for sperm motility. The SU and BPR lines show a different trend of motility across age categories compared to the BPR A line. The BPR C line shows a statistically significant increase in motility between age categories 2 and 3. The RIR line exhibits significantly higher motility at age categories 1 and 2 compared to the RIW and SU lines, although at age category 3, the differences are not statistically significant.

Figure 4 presents the interaction for the occurrence of head defects. The BPR C and RIW lines show completely different trends of the incidence of head defects. In the BPR C line, there is an increase in defects at age category 2, while in the RIW line, there is a decrease. These differences are statistically significant at age category 2, but not at age categories 1 or 3. The BPR A and BPR B lines exhibit consistently lower occurrences of head defects across all age categories when compared to the RIR and SU lines.

Figure 5 shows the influence of the interaction between line and age on the occurrence of tail defects. Due to high variability within each class of interaction, the incidence of tail defects between the groups is statistically insignificant in most cases. However, in the BPR C line, a lower incidence of tail defects was observed compared to the RIW line at age category 3.

Figure 6 illustrates the effect of the interaction between age and line on the percentage of normal spermatozoa. Similar to the previous graph, distinct patterns are observed between the RIW and BPR C lines. At Age 1 and Age 3, the RIW line shows statistically significantly lower percentages of normal spermatozoa compared to the BPR C line, while at Age 2, the values are nearly identical. In the BPR C line, there is a decrease in the percentage of normal spermatozoa at Age 2, whereas in the RIW line, the percentage increases during the same age category.

DISCUSSION

The semen volume observed in our study aligns with the values reported by other authors for laying hen lines (Machal and Krivanek 2002; Jarinkovicova et al. 2012; Tesfay et al. 2020), where the values for laying hen lines range from 0.52 ml to 0.66 ml. Higher values were reported by Londero et al. (2020), with average semen volumes of 1.21 ml in Rhode Island Red and White Plymouth Rock lines, but at an older age of the roosters. Similarly, broilers, particularly the Ross hybrid, also achieved comparable semen volumes (Tapeh et al. 2017; Ghadimi et al. 2024).

Our results show that the semen volume, regardless of the line, was highest at 43 weeks of age (0.48 ml) and then it decreased to an average of 0.41 ml at 52 weeks. The increase up to 34 weeks of age is consistent with another study of laying lines in the Czech Republic (Machal and Krivanek 2002), although no values were observed

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at the highest age category of 52 weeks. A study on White Leghorn and Beijing You chickens indicates a significant change in the semen volume with age, but with considerable variability between lines. In some lines, there is a decrease with age, while in others the change in the volume is not statistically significant (Shi et al. 2021). Similarly, in our study, the interaction between line and age for semen volume was statistically non-significant.

The sperm concentration and total sperm count (TSC) in our study were significantly different between the lines and age categories, and the interaction between line and age also had a statistically significant effect. Notable differences were observed between the BPR B and RIR lines. The difference between BPR and RIR lines was described in previous studies on laying hen lines in the Czech Republic (Machal and Krivanek 2002). The significant effect of the line factor was also reported in other studies (Jarinkovicova et al. 2012; Lukaszewicz et al. 2020).

However, in a study on White Leghorn and RIR the differences in sperm concentration between the lines were found to be statistically non-significant (Tesfay et al. 2020). In our study, the highest sperm concentration and TSC were observed at 43 weeks of age, followed by a decline at 52 weeks. This contrasts with a study examining the effect of age on semen quality in broilers, where the highest sperm concentration was reached at the youngest age category (26 weeks) (Tabatabaei et al. 2010).

Regarding sperm defects and motility, our findings are consistent with a number of other studies (Tapeh et al. 2017; Rahnama et al. 2020; Heng et al. 2022; Ghadimi et al. 2024). Sperm motility tends to be around 80% in both laying and broiler lines of chickens. In the study by Santiago-Moreno et al. (2018), a significant effect of the line on sperm motility was also reported, though the motility values in their study were lower compared to our findings. However, their study involved local breeds such as Black-Barred Andaluza and the combined Red-Barred Vasca line. In hybrids like ISA Brown and Hubbard Flex, lower motility values, of around 80%, were observed (Lukaszewicz et al. 2020). The non-significant effect of the line on specific morphological characteristics was described in a study involving RIR and White Plymouth Rock (Londero et al. 2020).

In contrast, our results point to a non-significant effect of the line only on tail defects. A similar non-significant effect on some morphological abnormalities was also mentioned in a study comparing laying and combined lines of chickens (Santiago-Moreno et al. 2018). Previous studies on laying lines like BPR, SU, and RIR in the Czech Republic indicated significant differences between these lines (Jarinkovicova et al. 2012).

In our study, the age was not found to have a significant effect on sperm defects, except for head and neck defects. Other morphological abnormalities varied with age at a significance level of 0.05. A study on broilers, divided into similar age groups (26, 34, and 45 weeks of age), demonstrated differences between age groups only for certain head defects (larger and smaller head) and coiled tail (Tabatabaei et al. 2010). However, in their study, defects of individual sperm parts were examined in more detail and divided into different subgroups.

The interaction between age and line exhibited significant variability for individual sperm morphological abnormalities. For head defects, there was a completely different pattern between BPR C and RIW lines. However, for the other parameters, BPR C and RIW lines showed similar trends. It is important to note that the RIW line was historically used in the creation of the BPR C line, which likely explains the similarities observed in some of the evaluated ejaculate parameters. Overall, there was considerable variability among the lines within the different age categories, and no uniform trend was observed across all parameters.

CONCLUSION

Based on the results of our study, it can be concluded that there are significant differences between lines and various age categories of roosters in terms of basic ejaculate parameters as well as selected morphological abnormalities. The line effect was significant for all the parameters examined, except for the frequency of tail defects. Age did not have a significant impact on sperm motility and the occurrence of head and neck defects. The interaction effect between age and line was significant for some parameters, with individual lines showing considerable differences across the age categories.

Regarding the interaction between age and line, there is a noticeable difference between lines at different ages, but some lines exhibit similar trends in many of the ejaculate parameters. This is partic-

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ularly evident for the BPR C and RIW lines. It is important to note that these lines were historically combined during their creation, particularly the RIW line, which contributed to the development of the BPR C line.

No clear trend of deterioration in basic ejaculate parameters and of the occurrence of morphological abnormalities with the increasing age of roosters was observed. Some defects, such as tail and acrosome defects, occurred more frequently in younger roosters, whereas immature spermatozoa were more common in older roosters. The overall proportion of normal spermatozoa was higher in the youngest age category.

Thus, it can be concluded that the results of this study highlight the necessity of monitoring the quality and quantity of rooster ejaculate indicators, not only for the selection of breeding lines but also for the production of hybrid layers. In newly created lines, a negative trend of ejaculate quality may manifest as reduced fertility of roosters, which subsequently affects the hatchability of chicks, not only in the original lines but also in final hybrid chicks. When increasing the efficiency of day-old chick production in hybrid laying hens, it is crucial to monitor the reproductive parameters in the breeding roosters, as well as take into account their age, which significantly affects the ejaculate parameters.

Conflict of interest

The authors declare no conflict of interest.

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