# Relationship of ejaculate quality and selected biochemical parameters of blood in cockerels of three laying lines

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**ABSTRACT**: Ejaculates and blood plasma were sampled from cocks of three laying lines: Barred Plymouth Rock (BPR), Sussex Light (SU), and Rhode Island Red (RIR). Ejaculates and blood plasma were sampled four times during the laying period of hens. The following ejaculate parameters were determined: sperm motility, concentrations of sperm cells, ejaculate volume. Sperm morphology was examined. In the blood samples, concentrations of glucose, cholesterol, creatinine, calcium, phosphorus, and magnesium were analysed. The motility of spermatozoa of the cocks was 47.0% (BPR), 47.7% (RIR), and 48.3% (SU), respectively. The highest volume of ejaculate was found in BPR line (0.66 $cm^3$), the lowest one in SU line (0.46 $cm^3$, $P \leq 0.01$); the highest sperm concentration was in SU line (2.46 × $10^6/mm^3$), the lowest one in RIR line (1.96 × $10^6/mm^3$, $P \leq 0.01$). The number of morphologically abnormal sperm cells was similar in all lines – 47.0% BPR, 47.7% RIR, and 48.3% SU, respectively. In general, the occurrence of defective spermatozoa was high in all three lines; the most frequent were tail defects (from 20.3 to 29.7%), while sperm cells with developmental anomalies were less frequent (only 0.3 to 0.4%). Calculated phenotypic correlation between sperm motility on the one hand and the occurrence of defective sperm cells on the other was negative ($r_p = -0.28$, $P \leq 0.01$), as well as the correlation between sperm motility and sperm numbers, and between sperm motility and ejaculate volume ($r_p = -0.28$, $P \leq 0.01$ and $r_p = -0.31$, $P \leq 0.01$, respectively). Negative correlations were found between the level of magnesium in blood plasma and numbers of morphologically defective spermatozoa in the ejaculate, defective heads and defective connecting pieces ($r_p = -0.33$, $P \leq 0.01$; $r_p = -0.23$, $P \leq 0.05$; and $r_p = -0.26$, $P \leq 0.05$). Level of magnesium was positively correlated to sperm motility ($r_p = 0.26$, $P \leq 0.05$). However, positive correlations existed between concentration of glucose in blood plasma of cocks and numbers of morphologically defective spermatozoa in sampled ejaculates ($r_p = 0.27$, $P \leq 0.01$). Our finding is in accordance with the results found in mammals and other animals, and it shows an important role of magnesium as a key contributor to the quality of ejaculate in aviary species, in our case in laying lines of domestic fowl (*Gallus domesticus*).

**Keywords**: cocks; ejaculate; blood plasma; pathological sperms; glucose; magnesium; biochemical criteria

In domestic fowl (*Gallus gallus* f. *domestica*), monitoring of both quantitative and qualitative parameters of ejaculates is an indispensable precondition of successful artificial insemination; on the basis of the obtained results it is then possible to select the individuals that produce sufficient amounts

Supported by the Internal Grant Agency AF MENDELU, Mendel University in Brno, Czech Republic (Project No. TP 8/2011).

of good-quality semen. There are many methods how to evaluate ejaculate quality and estimate the fertilisation potential of spermatozoa; some of them are rather subjective while others require special laboratory equipment. Ejaculate volume is an important parameter of semen quality, similarly the motility of sperm cells and concentration of spermatozoa in ejaculates. A direct progressive movement is an indicator of the functional quality of the sperm cell. Máchal et al. (1996) mentioned that in cocks the values of spermatozoa motility ranged from 62.4 to 88.3% and the ejaculate volume ranged from 0.16 to 0.80 $cm^3$. Both parameters changed in dependence on age and the breeding line. Froman and Feltmann (2000) mentioned that in domestic fowl concentrations of motile spermatozoa ranged from 0.52 to 0.95 × $10^6$/ml.

A morphological examination of spermatozoa is one of important qualitative parameters of ejaculates. An increased percentage of morphologically modified sperm cells may result in impaired fertility (Saacke et al., 2000). A decrease in fertility and the occurrence of pathological sperm cells may be markedly influenced not only by chemical and physical factors but also, and above all, by stress and age of males.

Serum biochemical profile has been used in several species of domestic livestock to monitor herd health. Application of this technique to commercially raised poultry flock has been limited by a lack of suitable reference ranges for most of the parameters tested although much work has been done on specific individual parameters. Haematological and biochemical status is a reflection of many factors such as sex, age, breed, diet, management, and stress level. Among the most important biochemical parameters plasma triglycerides and cholesterol, as well as glucose, creatinine, and minerals like calcium, phosphorus, and magnesium are included. Blood plasma cholesterol level in cocks of laying lines ranges from 2.33 to 2.75 mmol/l, glucose in laying hens lines is between the values of 4.87 to 15.15 mmol/l (Máchal, 1999). Silva et al. (2007) revealed content of creatinine in blood plasma in ranges from 46 to 59 µmol/l. Concentrations of plasma calcium in broiler chicken was from 2.52 to 3.80 mmol/l, phosphorus from 2.78 to 5.27 mmol/l, and plasma magnesium from 0.83 to 0.84 mmol/l (Večerek et al., 2002).

Magnesium is an element that is necessary for the function of dynein-ATPase, intracellular motor for sperm motility (Vívenes et al., 2009). The literature about the effects of magnesium concentration on sperm parameters refers mostly to other than avian species. In humans, sperm cell motility was enhanced by 0.2–1.0mM of $Mg^{2+}$, while higher concentration led to the same motility compared to control levels (Magnus et al., 1990). In sea urchin, as well as in humans, $Mg^{2+}$ has been found to increase viability of spermatozoa in dose-dependent manner (Dawson et al., 1998). Enhancing effect of $Mg^{2+}$ ions of seminal plasma on motility of spermatozoa was also found, among others, in fish (Hajirezaee et al., 2010; Bozkurt et al., 2011).

The aim of this study was to compare qualitative and quantitative parameters of ejaculates, as well as selected biochemical parameters of blood plasma collected from cocks of three lines of laying hens. Furthermore, relationships between these parameters regardless of the line were evaluated.

## MATERIAL AND METHODS

Ejaculates and blood plasma were sampled on a breeding farm from cocks of three laying lines of the following breeds: Barred Plymouth Rock (BPR), Sussex Light (SU), and Rhode Island Red (RIR). Lines of these breeds are used when creating hybrid combinations of layers. At the age of 16 weeks, cockerels were transferred from the poultry-rearing facility into a hall with cage technology for adult cocks. Each cock was placed into one cage (2025 $cm^2$ per cock) and fed a complete feed mixture with a quantitative restriction of 120 g per day. Drinking water was supplied with nipple drinkers. The light regime was 12/12 h of light and dark per day.

Cocks were divided into three groups according to line, including 21 males from each line. The semen sampling was performed four times, at the age of 173, 239, 315, and 391 days. Blood was sampled from *vena cutanea ulnaris* immediately after semen collection and diluted with an anticoagulant (Heparin).

Ejaculates were sampled by means of a dorso-abdominal massage (Burrows and Quinn, 1937). Immediately after semen collection the following spermatological parameters were estimated: ejaculate volume, sperm motility, and concentration of spermatozoa. Ejaculate volume was determined by means of a calibrating pipette. Sperm motility was estimated subjectively under the microscope. Concentration of spermatozoa was estimated also by the haemocytometric method. Ejaculates were

diluted in 0.5% saline solution (dilution ratio 1 : 5) to evaluate their morphological abnormalities. In each sample, the total number of 200 spermatozoa was evaluated with regard to the following morphological alterations: occurrence of developmental anomalies, numbers of degenerated spermatozoa, changes in the head, changes in the midpiece, and changes in the tail; frequencies were expressed in percents. The following alterations were classified among head changes: the heads too small or too big, twisted heads, curved heads, missing heads; alterations of the midpiece were characterized as its narrowing, widening and/or distortion, and tail changes involved its twisting, bending and/or absence. All spermatozoa with developmental anomalies were classified as degenerated. An objective evaluation of the shape and variability of spermatozoa was performed for example by Severa et al. (2010) who studied also the occurrence of morphologically changed spermatozoa.

Blood samples were centrifuged (3000 rpm for 10 min), and the obtained blood plasma was used for biochemical analyses. Blood analyses were performed using BIO-TEST sets (Erba Lachema s.r.o., Brno, Czech Republic). Concentrations of the following compounds and elements were estimated in blood plasma of experimental cocks: glucose, cholesterol, creatinine, Ca, P, and Mg.

Statistical analysis was performed using the software package Statistica Vers. 8.0. Differences between the lines of cocks were evaluated using one-way ANOVA with Tukey's post-hoc test. Characteristics under study were expressed as means ± standard deviations. Calculated phenotypic correlations (Pearson's correlation coefficients) were used for the evaluation of the dependence between ejaculate parameters and biochemical characteristics of blood plasma.

## RESULTS AND DISCUSSION

Mean values of both qualitative and quantitative parameters of ejaculates collected from cocks originating from three laying lines of BPR, SU, and RIR chicken breeds as recorded in the course of the production period are presented in Table 1. The maximum and the minimum average ejaculate volumes (0.66 and 0.46 $cm^3$, respectively) were found in the lines BPR and SU. These values are lower compared to those found in the study of Siudzińska and Lukaszewicz (2008), and higher in comparison with the results of Máchal and Jankowski (2002) in a laying line of RIR cocks (0.87 $cm^3$). As far as the motility of spermatozoa was concerned, the recorded values were very similar and ranged from 62.7 to 68.4%. There were no statistically significant differences between these values. Máchal et al. (1996) mentioned relatively high percentages of motility in a paternal line of RIR breed (85.5 to 88.3%). In our study, the lowest mean concentration of spermatozoa in ejaculates

Table 1. Qualitative and quantitative parameters of ejaculate in cocks of initial laying lines in the course of the production period ($\bar{x} \pm S_{\bar{x}}$)

| | | BPR ($n$ = 82) | SU ($n$ = 81) | RIR ($n$ = 83) |
|---|---|---|---|---|
| Ejaculate volume ($cm^3$) | | $0.66^{A} \pm 0.22$ | $0.46^{B} \pm 0.17$ | $0.55^{C} \pm 0.16$ |
| Sperm motility (%) | | 67.9 ± 27.7 | 68.4 ± 28.4 | 62.7 ± 31.2 |
| Sperm concentration ($10^6/mm^3$) | | $2.45^{A} \pm 0.64$ | $2.46^{A} \pm 0.94$ | $1.96^{B} \pm 0.84$ |
| Morphological changes of spermatozoa (%) | head | $1.8^{a} \pm 3.6$ | $1.3^{b} \pm 1.9$ | $3.2^{c} \pm 4.0$ |
| | connecting piece | $15.1^{a} \pm 9.7$ | $18.5^{b} \pm 9.7$ | $23.9^{b} \pm 15.2$ |
| | tail | 29.7 ± 11.0 | 28.1 ± 9.0 | 20.3 ± 8.7 |
| | degenerated spermatozoa | 0.4 ± 0.7 | 0.3 ± 0.8 | 0.4 ± 0.8 |
| | total changes | 47.0 ± 14.6 | 48.3 ± 13.9 | 47.7 ± 15.6 |
| Normal spermatozoa (%) | | 53.0 ± 14.6 | 51.7 ± 13.9 | 52.3 ± 15.6 |

BPR = Barred Plymouth Rock, SU = Sussex Light, RIR = Rhode Island Red

$^{a–c}$statistically significant ($P \leq 0.05$) differences were found among values with different letters

$^{A–C}$statistically significant ($P \leq 0.01$) differences were found among values with different letters

Table 2. Selected biochemical parameters of blood plasma in cocks of initial laying lines in the course of the production period

| | BPR | | SU | | RIR | |
|---|---|---|---|---|---|---|
| | *n* | $x \pm S_x$ | *n* | $x \pm S_x$ | *n* | $x \pm S_x$ |
| Glucose (mmol/l) | 69 | $13.64^{A} \pm 1.60$ | 69 | $13.46^{A} \pm 1.79$ | 71 | $12.41^{B} \pm 1.60$ |
| Cholesterol (mmol/l) | 67 | $2.67^{A} \pm 0.71$ | 65 | $2.99^{B} \pm 0.66$ | 71 | $2.81 \pm 0.45$ |
| Creatinine (μmol/l) | 26 | $53.05 \pm 6.85$ | 39 | $54.48 \pm 6.75$ | 35 | $52.08 \pm 6.51$ |
| Calcium (mmol/l) | 67 | $1.93^{a} \pm 0.35$ | 57 | $1.90^{a} \pm 0.46$ | 65 | $2.15^{b} \pm 0.65$ |
| Phosphorus (mmol/l) | 67 | $3.96 \pm 1.94$ | 65 | $4.84^{A} \pm 3.06$ | 66 | $3.31^{B} \pm 1.61$ |
| Magnesium (mmol/l) | 67 | $1.15 \pm 0.29$ | 64 | $1.18 \pm 0.29$ | 66 | $1.08 \pm 0.21$ |

BPR = Barred Plymouth Rock, SU = Sussex Light, RIR = Rhode Island Red

$^{a,b}$statistically significant ($P \leq 0.05$) differences were found among values with different letters

$^{A,B}$statistically significant ($P \leq 0.01$) differences were found among values with different letters

was found in the line RIR ($1.96 \times 10^6$/mm$^3$). On the other hand, in lines BPR and SU, mean spermatozoa concentrations were higher and ranged from 2.45 to $2.46 \times 10^6$/mm$^3$; the values obtained in our study are lower compared to those found by Siudzińska and Lukaszewicz (2008) in cocks of laying lines (3.18 to $4.69 \times 10^6$/mm$^3$). The occurrence of all morphologically changed spermatozoa in ejaculates of cocks of BPR, SU, and RIR lines was not markedly different and ranged from 47.0 to 48.3%; the observed differences were statistically non-significant. Fraction of morphologically abnormal spermatozoa is similar to the results of Siudzińska and Lukaszewicz (2008) (33.1 to 44.5%) in cocks of laying breeds. The highest occurrence of morphological malformations was observed in tails (20.3% in RIR, 28.1% in SU, and 29.7% in BPR). Lukaszewicz et al. (2008) reported the highest percentage of morphological defects in heads of spermatozoa of cocks of the laying breed Italian Partridge; depending on method of dyeing, these values ranged from 11.0 to 14.3%. When using the dyeing method published by Blom (1981), Lukaszewicz et al. (2008) observed a high number of head deformations (76.1%). The highest and the lowest numbers of spermatozoa with defect heads were found in cocks of the lines RIR and SU (3.2%, $P \leq 0.01$ vs. 1.3%, $P \leq 0.05$, respectively). However, the occurrence of developmental anomalies and degenerated spermatozoa was relatively low (0.4% in lines RIR and BPR, 0.3% in line SU).

Mean values of blood plasma biochemical parameters of cocks of individual laying lines are presented in Table 2. The lowest and the highest mean concentrations of glucose in blood plasma were found in cocks of the RIR and BPR laying lines (12.41 and 13.64 mmol/l, $P \leq 0.01$, respectively). In the SU line this concentration was also high (13.46 mmol/l). Blahová et al. (2007) observed concentrations of glucose from 13.01 to 13.73 mmol/l in the male of Ross 308. The lowest concentration of cholesterol, which is an indispensable precursor for synthesis of many important steroid hormones, was 2.67 mmol/l in BPR line. Higher values of plasmatic cholesterol were observed by Tůmová et al. (2004) in laying hens. The level of creatinine in blood plasma was another parameter involved in our study because in poultry it reflects the supply of N-compounds. Mean concentrations of creatinine ranged from 52.08 to 54.48 μmol/l and were not affected by line. Mean values of plasmatic mineral compounds: the concentration of calcium was significantly influenced by line (1.90–2.15 mmol/l, $P \leq 0.05$). The highest value was in RIR and the lowest in SU. However, there was no effect of line on phosphorus (3.31–4.84 mmol/l, $P \leq 0.01$) and magnesium (1.08–1.18 mmol/l) concentrations. Blood plasma analyses of the content of mineral substances were also observed by Večerek et al. (2002) in broiler chickens with similar results.

Estimated phenotypic correlations existing between qualitative and quantitative ejaculate parameters and morphological changes of spermatozoa are presented in Table 3. Calculated phenotypic correlations between motility and occurrence of morphologically changed spermatozoa were neg-

Table 3. Phenotypic correlation assessment between the cocks of initial laying lines and qualitative and quantitative parameters of cock ejaculate

| | Sperm concentration | Ejaculate volume | Morphological changes of spermatozoa | | | | | Normal spermatozoa |
|---|---|---|---|---|---|---|---|---|
| | | | head | connecting piece | tail | degenerated spermatozoa | total morphological changes | |
| Sperm motility | −0.28[A] | −0.31[B] | −0.24[a] | −0.38[C] | 0.12 | −0.12 | −0.28[D] | 0.28[E] |
| Sperm concentration | | 0.09 | −0.02 | 0.00 | −0.09 | −0.02 | −0.08 | 0.08 |
| Ejaculate volume | | | 0.08 | 0.04 | 0.02 | 0.20 | 0.08 | −0.08 |

[a]statistically significant correlation ($P \leq 0.05$)
[A–E]statistically significant correlation ($P \leq 0.01$)

ative ($r_p$ = −0.28); this means that the lower the occurrence of morphologically abnormal spermatozoa, the higher their motility. Similarly, negative correlations existed between motility and concentration of spermatozoa on one hand and ejaculate volume on the other ($r_p$ = −0.28 and −0.31). The correlation between motility and the occurrence of pathological heads was negative and statistically insignificant. Insignificant positive correlation was observed between ejaculate volume and various morphologically changed spermatozoa ($r_p$ = 0.02 to 0.20).

Phenotypic correlations found between qualitative and quantitative ejaculate parameters on the one hand and biochemical parameters on the other are presented in Table 4A. There was a negative phenotypic correlation between motility and concentration of glucose in blood plasma ($r_p$ = −0.27). A significant negative correlation was found also between creatinine level in blood plasma and concentration of spermatozoa in cock's ejaculate ($r_p$ = −0.20, $P \leq 0.05$). On the other hand, a significant positive phenotypic correlation existed between motility and Mg concentration in blood plasma ($r_p$ = 0.26, $P \leq 0.05$). We also observed insignificant negative phenotypic correlations between the concentration of spermatozoa and ejaculate volume ($r_p$ = −0.06) and between the concentration of

Table 4. Phenotypic correlation assessment between (A) qualitative and quantitative parameters, (B) presence of morphological changes of spermatozoa and selected biochemical parameters of blood plasma in cocks

| | Glucose | Cholesterol | Creatinine | Calcium | Phosphorus | Magnesium |
|---|---|---|---|---|---|---|
| **(A) Qualitative and quantitative parameters** | | | | | | |
| Sperm motility | −0.27[A] | 0.00 | 0.08 | −0.04 | −0.09 | 0.26[c] |
| Sperm concentration | 0.26[a] | −0.06 | −0.20[b] | 0.06 | 0.07 | −0.15 |
| Ejaculate volume | 0.26[B] | −0.15 | −0.03 | 0.04 | −0.04 | −0.18 |
| **(B) Presence of morphological changes of spermatozoa** | | | | | | |
| Head | −0.09 | −0.09 | −0.13 | 0.10 | 0.12 | −0.23[b] |
| Connecting piece | 0.12 | 0.11 | −0.15 | −0.05 | 0.00 | −0.26[c] |
| Tail | 0.23[a] | −0.05 | 0.17 | −0.01 | −0.01 | −0.08 |
| Degenerated spermatozoa | 0.12 | 0.18 | −0.03 | 0.17 | 0.04 | −0.13 |
| Total morphological changes | 0.27[A] | 0.04 | −0.02 | −0.02 | 0.02 | −0.33[C] |
| Normal spermatozoa | −0.27[B] | −0.04 | 0.02 | 0.02 | −0.02 | 0.33[D] |

[a–c]statistically significant ($P \leq 0.05$)
[A,B]statistically significant ($P \leq 0.01$)

spermatozoa and the level of cholesterol in blood plasma ($r_p$ = –0.15).

Estimates of phenotypic correlations existing between the occurrences of morphologically changed spermatozoa and individual biochemical parameters of blood plasma are presented in Table 4B. Particularly interesting are significantly negative correlations which were found between the level of Mg in blood plasma and morphologically abnormal heads of spermatozoa ($r_p$ = –0.23, $P \leq 0.05$) and abnormal connecting piece of spermatozoa in ejaculates ($r_p$ = –0.26, $P \leq 0.05$); this means that the higher the Mg level in blood plasma, the lower the occurrence of pathologically changed spermatozoa and the higher the occurrence of morphologically normal ones. Positive effect on sperm cell motility can be attributed to dynein-ATPase, molecular motor of the sperm cell, which is dependent on $Mg^{2+}$. Sperm cell motility also significantly decreased with increasing concentration of blood glucose, the effect may be attributed to reactive oxygen species formation in hyperglycaemia (Amaral et al., 2006).

The fact that sperm motility of cocks is in a negative relation to other parameters of ejaculate quality, but also to the frequency of occurrence of morphologically abnormal sperm cells can therefore be confirmed. Sperm concentration and ejaculate volume do not seem to be significantly influenced by the plasmatic level of magnesium. Because of the identified negative correlations between concentration of magnesium in blood plasma of cockerels and frequency of morphologically defective spermatozoa (changes on heads and connecting pieces) of cocks and a positive relationship between magnesium concentration and sperm motility, it is useful to take care to the body saturation of magnesium during the production period of cockerels.

Our finding is in accordance with the results found in mammals and other animals, and it shows an important role of magnesium as a key contributor to the quality of ejaculate in aviary species, in our case in laying lines of domestic fowl (*Gallus domesticus*).

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Received: 2012–01–18
Accepted after corrections: 2012–03–30

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