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EFFECT OF LONG-TERM COLLECTION FREQUENCY ON THE SEMEN TRAITS IN PLYMOUTH ROCK ROOSTERS

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Abstract

In this study, the effect of ejaculate sampling frequency on the morphological properties of sperm in Plymouth Rock roosters was determined. Four experimental groups were tested: of five, four, three, and two weekly ejaculates. The age of the roosters at the beginning of the experiment was 28 weeks and the duration of the experiment was 30 weeks. Increasing the frequency of semen sampling from one weekly sampling to five samplings per week had the following effects: 1. increase in ejaculate volume, but decrease in sperm density; 2. no significant changes in sperm motility; 3. increase of spermatozoa viability and 3. significant influences on the percentage of anomalies but no significant influences on the types of anomalies (the most common being the sperm head abnormalities).

Key words*: semen feature, spermatozoa abnormality, rooster.*

INTRODUCTION

Sperm morphology and morphometry were considered important techniques in the evaluation of sperm, on the occasion of the development of artificial insemination techniques and technologies. However, sperm traits and morphometry data were reported for only a few bird species, most of which were recorded for roosters, ducks, turkeys and quails. Recently, some studies have focused on wildlife (Santiago‐Moreno et al., 2016).

The intention of breeders is to use as much as possible the breeding male parents or grandparents of broiler or laying hens. A huge chapter of poultry farming has opened and developed with the emergence and expansion of artificial insemination (Bunaciu et al., 1978; Bunaciu et al., 1992). Their use has not been without effect on the physiology of the males from which the semen is collected as well as on the composition of the sperm, although the advantages of using artificial insemination in birds are unquestionable. Here are some of them: 1. reducing the number of males; 2. the possibility of improving the selection and breeding works and 3. There are indisputable economic advantages resulting from the reduction of the number of males and from the increase of the biological value of the poultry material as a result of the facilitation of the selection and breeding works (Bunaciu et al., 2009). As much as the rooster was to be protected for use in artificial insemination, the method of harvesting and the frequency of harvesting altered the processes of spermatogenesis and subsequently the biological composition and properties of sperm (Noirlaut and Brillard, 1999; Riaz et al., 2005; Racha et al., 2015).

The aim of this study was to determine the effects of different sperm sampling (ejaculates) frequencies on ejaculate volume, motility, density, viability and abnormality of spermatozoa, in roosters.

MATERIALS AND METHODS

The biological material was represented by Plymouth Rock roosters aged 28 weeks and reared in an extensive system, in individual cages 60 x 60 x 80 cm, housed in a room with a temperature of 20-28°C. The birds had free

access to forage and water and were fed on a standard commercial diet (main ingredients by %: wheat 41.9, barley 31.1, oats 11.8, soybean meal 5.5, grass meal 2.5, fish meal 5.5, dicalcium phosphate 0.5, limestone 0.8, vitamin and mineral premix 0.5). The light program was 15 hours light and 8 hours dark. Semen was collected using the method described by Bunaciu et al. (2009). The experiment was organized in 4 experimental (five roosters each) variants depending on the regime of use of breeding roosters and lasted 30 weeks, according to the following scheme:

Variant	Days between ejaculates	Day of the week						
		Mo	Tu	We	Th	Fr	St	Sa
V1	2	E	E	E	E	E	No	No
V2	3	E	E	No	E	E	No	No
V3	4	E	E	No	E	No	No	No
V4	5	E	No	No	E	No	No	No

Legend: Mo-Monday; Tu - Tuesday; We - Wednesday; Th - Thursday; Friday; ST -Saturday; Sa – Sunday; E - ejaculate; No - break day

The ejaculates were collected in transparent glass graduated collection tubes; volumes were recorded directly in the tube immediately after collection at the lower margin of the semen meniscus and are expressed in µL. Sperm motility was assessed by a wet preparation technique using a Nihon Kohden optical microscope (Sapaco 2000, Bucharest, Romania) on a warmed plate. Motility was estimated by direct observation of spermatozoa in at least five fields, using 400 × magnification and a lowered condenser to disperse the light. Motility is expressed here as the percentage of all spermatozoa showing progressive movements. Nonprogressive spermatozoa with other patterns of movement were not considered in this category. Sperm count was determined using a hemocytometer with a Nihon Kohden optical microscope. For this assessment, fresh semen samples were diluted (1:200) and fixed using neutral Hancock's solution, (62.5 mL of 37% formaldehyde, 150 mL of 1% saline, 150 mL of sodium phosphate buffer, and 500 mL of double-distilled water) and a Potain pipette. The results are expressed as the number of spermatozoa per mL. Viability of the spermatozoa was evaluated by eosin-nigrosin staining (Merck, Darmstadt, Germany) according to Kondracki et al. (2017). The results are expressed as the percentage of all spermatozoa classed as viables.

The data obtained were centralized using the Excel 2010 program and the statistical processing was performed using the GraphPad program for Windows, version 8.0.2, GraphPad Software, Inc. The correlations of the frequency of ejaculates and the sperm features were analysed by Pearson correlation coefficient. The significance between the groups was analysed by ANOVA and the the differences were considered significant for values of $P \leq 0.05$.

RESULTS AND DISCUSSIONS

The volume of ejaculate ranged between the limit values of 0.32 (V1) and 0.25 mL (V4, which represents a percentage decrease of 28%), the highest value being recorded for the group of roosters with five ejaculates per week and the lowest in the group of roosters with two ejaculates per week. The differences between the groups are significant and the decrease in ejaculate volume is strongly correlated with the number of ejaculations ($r = +0.96$). It can be seen that a higher frequency of sperm collection stimulates the increase of sperm volume, probably by increasing testicular vasodilatation and extravasation of a higher volume of water in the seminiferous tubules. The analysis of the following sperm parameters will allow the identification of the effects upon the spermatogenesis and sperm properties.

Sperm density showed an upward evolution, increasing from 1.22×10^9 (in V1) to 1.68×10^9 sperm per mL of sperm (in V4), which represents a percentage increase of 37.7%). The differences between the groups were significant ($P = 0.030$). The increase was not linear, the variant of group V3 presenting a lower value than the adjacent groups. Sperm density evolved inversely with the number of ejaculates. However, it correlates with sperm volume, the increase in ejaculate volume being based on the increase in seminal plasma volume, not on the intensification of the spermatogenesis process. This sperm parameter correlated weakly and negatively with the number of ejaculates: $r = -0.122$.

The percentage of motile sperm does not seem to be influenced by the number of weekly ejaculations: the values ranged between 55.5 (in

V4) and 57.0% (P = 0.244). Obviously this sperm parameter did not correlate with ejaculate frequency: r = 0.082.

Sperm viability was a parameter significantly altered by ejaculation frequency: P = 0.053. The values ranged from 90.52% for variant V1 with five ejaculations per week to 88.8% for variant V4 with two ejaculations per week. This parameter of Plymouth Rock rooster sperm was negatively correlated with ejaculation frequency: r = 0.076.

The percentage of abnormal sperm decreased from 99.9% to 84.4% in the four experimental variants, the differences between the variants proving to be significant: P = 0.032. This decrease was also correlated with the number of weekly ejaculations: r = +0.767.

The results obtained by us on the mentioned sampling variants are generally superposable with those reported by Schramm (2005) on Plymouth Rock roosters in two different experimental variants of frequency of sperm collection. The author shows the differences in sperm quality from roosters with three, respectively, five weekly sperm harvests. Thus, the volume of ejaculate increased as the frequency of sampling increased while the density of sperm decreased accordingly, which reveals an intensification of sperm fluid secretions (as long as they exist in birds). Sperm motility and viability were found significantly altered while the percentage of sperm with abnormalities increased significantly due to an intensification of the spermatogenesis process. Riaz et al. (2004) determined the characteristics of sperm including motility, volume, concentration and number of sperm per ejaculate in Hubart roosters subjected to a regimen of increased sperm sampling frequency. Sperm motility was not affected by the collection interval, but sperm volume was smaller. Sperm concentration also decreased significantly with increasing sampling frequency. Mkpughe and Bratte (2015) determined the effect of breed and sperm sampling frequency on indigenous Nigerian roosters: sperm concentration and sperm count per ejaculate were the only attributes of sperm affected by ejaculation frequency and increased significantly (P <0.05) with increasing frequency of ejaculation, which is not in agreement with our results but can be interpreted as a stimulation of the spermatogenesis process.

The effects on sperm morphology in our study were analyzed at the end of the experimental period, respectively in the last week of sperm collection. The results of this study are summarized in Table 1. Among the types of sperm abnormalities were: twisted sperm, localized abnormality in the head, followed by bent sperm, cytoplasmic drop sperm (immature sperm, Figure 1), and broken sperm (without acrosome and tail). Regarding the percentage of sperm with abnormalities, the analysis of the data presented in Table 2 shows that the percentage of abnormal sperm (calculated on the total sperm examined) varied between a minimum of 13.12% in V2 and 14.12% in V3. For the other variants, the values were very close: 13.66% in V1 and 13.84% in V4, respectively). Statistical analysis of differences between groups showed no significant values (P<0.05). Head abnormalities were most common.

Table 1. Comparative presentation of the sperm traits from roosters subjects of different weekly ejaculates

Item	UM	Number of ejaculates per week				SD	P
		V1 (five ejaculates)	V2 (four ejaculates)	V3 (three ejaculates)	V4 (two ejaculates)		
Ejaculate volume	mL	0.32^{b}± 0.01	0.32± 0.03	0.28± 0.07	0.25^{b}± 0.07	0.04	0.044
Spermatozoa density	$x10^{9}$ /mL	1.22^{c}± 0.34	1.55± 0.23	1.20± 0.21	1.68^{c}± 0.54	0.05	0.030
Spermatozoa motility	%	57.0± 4.5	55.9± 3.0	55.2± 3.2	55.5± 4.0	2.27	0.244
Spermatozoa viability	%	90 .52± 5.89	90.9± 4.87	89.0± 5.08	88.8± 3.09	4.21	0.053
Normal spermatozoa	%	88.9^{a}± 11.0	88.7± 9.09	86.0± 13.3	84.4^{a}± 14.0	6.46	0.032

The values are mean ± standard error of mean.
Each value represent the mean of 4-5 ejaculated samples
Values with the same superscript on the same row differ significantly
SD = standard deviation
P calculated by Tukey test

An important study on the effect of sperm harvesting frequency on roosters on sperm morphology was undertaken by Noirault and Brillard (1999) in turkeys (study lasting 10 weeks, from 30 to 40 weeks).

Table 2. The effect of ejaculate frequency on the type and frequency of spermatozoa abnormalities in Plymouth Rock roosters (percentage of sperm with abnormalities in the total sperm examined)

Type of anomaly	V1	V2	V3	V4	Total (%)
1- cytoplasmic drop (immature)	1.44	1.14	1.24	1.18	5.00
2- boselates (acrosome anomaly, head and intermediate piece)	1.06	1.11	1.05	1.18	5.40
3- bosses (anomaly in the head and the intermediate piece)	1.07	1.1	1.06	1.01	5.24
4- twist, head anomaly	1.65	1.74	1.76	1.79	7.94
5- whip, anomaly at the intermediate piece	1.17	1.33	1.11	1.13	5.74
6- club (anomaly at the acrosome, head and intermediate piece	1.05	1.03	1.11	1.09	5.28
7- rosette (acrosome abnormality, head, intermediate piece, and tail)	1.01	1.03	1.05	1.04	5.13
8- long (anomaly in the head and the intermediate piece)	1.03	1.01	1.01	1.01	5.06
9- bend (anom. at the head)	1.4	1.38	1.61	1.58	6.97
10- broken	1.01	1.01	1.01	1.01	5.04
11- vacuolised	1.03	1.01	1.01	1.01	5.06
Percentage of total types of abnormalities	13.66	13.16	14.12	13.84	

Note: Values are presented as mean

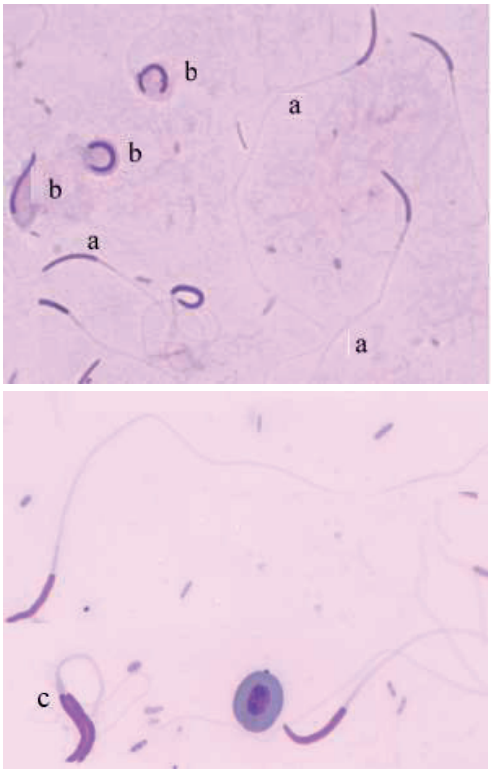

Figure 1. Spermatozoa abnormalities in rooster: a- normal; b- protoplamsmatic dropplets; c- double headed

The turkeys were distributed in groups from which semen was collected once every two weeks, weekly, twice a week, three times a week, five, six and seven times a week (respectively).

Apparently paradoxically, the authors conclude that turkeys produce better quality sperm, parametrically speaking, when the harvesting frequency is higher than when the harvesting frequency is lower. The results obtained by us on Plymouth Rock roosters are thus consistent with those reported by these authors in turkey. Malecki et al. (1998) investigated the effects of sperm sampling frequency on emu sperm morphology: emu sperm is characterized by a high sperm denial. However, the increase in ejaculation frequency did not affect their morphology. A study on the impact of harvest frequency on sperm traits was conducted by Rakha et al. (2015) on Red Jungle hen (*Gallus gallus murghi*), an endangered species native to South Asia: the number of live sperm cells was higher ($P>0.05$) in the case of short-range harvests (12 hours), compared to 24, 48 and 72. The research carried out by Bunaciu et al. (1978) on three turkey breeds: White large type, White small type and Bronze breed showed that the percentage of dead and abnormal sperm can increase as the males get older, respectively in the breeding season. Marques and Ogasawara (1974) in a study on turkey semen showed that in this species, the semen, yellow in color, contains a large number of abnormal sperm compared to roosters, in correlation with a lower ability to fertilize semen. Our research reveals differences in rooster sperm abnormalities compared to other species as well as the weak influence of ejaculations on this aspect of sperm. Values of the evolution of the normal percentage of sperm in the ejaculate were

revealed by Siudzinska and Lukaszewicz (2008) in a study performed on several cock breeds of different sizes. Three of these breeds did not show significant differences in the percentage of normal spermatozoa in the sperm (values between 70.5 and 69.1, which is in agreement with the results of our research). Only one breed, the Italian Partridge, which is a light-sized breed, had a much lower percentage, at 54.0%.

CONCLUSIONS

Our study on Plymouth rock roosters subjected to a sperm sampling regimen 5 to 2 times a week revealed changes in the type and frequency of different types of abnormalities. Increasing the frequency of ejaculation to five per week does not negatively affect the mor- phological properties of sperm, which can be taken into account in determining the use of these roosters.

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