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Research Article

Semen Collection from Japanese Quail (*Coturnix japonica*) Using a Teaser Female

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Abstract: The quantity and quality of Japanese quail (*Coturnix japonica*) semen collected individually after stimulation by a teaser female was assessed. From among 25 mature males, 10 were selected for experimental purposes on the basis of reaction intensity to stimulation, size of the proctodeal foam gland, and ejaculation speed. Males were kept individually in cages (32 × 44 × 24 cm) and 5 females that served for male stimulation were kept in a group cage (60 × 45 × 48 cm). The applied method of semen collection was very effective and with it we obtained ejaculates of good quality; average volume of ejaculate collected was 27 µl (range: 20-35 µl per individual). Spermatozoa concentration for the entire experimental period and all males averaged 693×10^6 ml^{-1} (range: 592 to 812×10^6 ml^{-1}). The sperm quality factor averaged around 16 (range: 11.5-19.2). The total amount of live spermatozoa was high (range: 87.6%-95.2%; mean: 94%), while the number of live, morphologically normal cells varied between individuals from 77.1% to 86.4% (mean: 83.4%). Among the live deformed cells, macrocephalic (2.8%) and bent-neck (1.6%) were the most frequently observed deformities.

Individual differences in reaction to the semen collection procedure, as well as the quality and quantity of fresh semen were observed. The high level of quantitative and qualitative traits of the quail semen collected after stimulation of the male with a teaser female suggests that this method can be recommended in experiments on quail reproduction.

Key Words: Japanese quails, semen collection, semen characteristics

Introduction

The Japanese quail, which belongs to the family *Phasianidae* (order *Galliformes*), along with chickens, partridges, and pheasants, is one of the most popular bird species, and is extensively used not only for eggs and meat production, but also as a pet and laboratory animal. Both quail meat and eggs are characterised by high nutritive value. Moreover, in comparison to other poultry species they have many advantages as a pilot and model subject in poultry studies. Adult birds are relatively small, their body weight ranges between 100 and 200 g, and daily food intake from ranges from 20 to 30 g per bird. Females begin to lay eggs at the age of 6 weeks, and during the entire reproductive period, lasting from 10 to 12 months, are able to lay about 300 eggs. The incubation period, lasting 17-18 days, is also shorter than in other poultry species and 4 generations per year can be obtained.

Thus, the ease of maintenance, reduced feeding costs, short incubation period and rapid generation turnover make quails a model animal for experiments on avian genetics, endocrinology, embryology, physiology, and nutrition. Quails can be also used in experiments aimed at evaluating different biotechnological methods of bird reproduction.

A simple and effective method of semen collection and insemination is necessary in order to make use of the potential role of quails in poultry research and production.

Dorso-abdominal massage, as described by Burrows and Quinn (1) for chickens and turkeys, is the most frequently used method of semen collection in birds (2-4). Wentworth and Mellen (5) adopted this method for semen collection from quails and later several modifications of the method were elaborated by other

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researchers (5-9). However, our preliminary experiments (10) on quail semen collection indicated that the dorso-abdominal massage method is insufficient, resulting in small volumes of ejaculate (15 µl on average) and low spermatozoa concentration (606×10^6 ml^{-1}). In birds semen can also be effectively collected by male stimulation using a teaser female. The later method was successfully used by Chełmońska et al. in order to obtain semen from turkeys (11) and Muscovy drakes (12), and it is commonly used in practice for Mule duck production.

Therefore, the aim of the present experiment, carried out at the Department of Poultry Breeding, Wroclaw University of Environmental and Life Sciences, Poland, was to evaluate the effectiveness of female stimulation of the male as a method for semen collection from Japanese quails. Observations of male behaviour and their response to the semen collection procedure were carried out simultaneously, and the quantitative and qualitative characteristics of ejaculates were evaluated.

Materials and Methods

The study included 25 male Japanese quail and 5 females that served as male stimulation. Males were kept in individual cages ($32 \times 44 \times 24$ cm) and females in a group cage ($60 \times 45 \times 48$ cm) at room temperature (22 °C). During the reproductive period birds were subjected to a 14-h/10-h light/dark cycle. Water and feed were available ad libitum and supplemented with minerals and vitamins. The mixture contained 11.7 MJ of metabolisable energy and 220 g of crude protein per kilogram. Daily feed consumption averaged 30 g per bird.

The experiment was divided into 2 stages. During the first stage, a group of 25 sexually mature males (10-11 weeks of age) were subjected to 6 training massages designed to evaluate their reaction to the semen collection procedure. Males were massaged 3 times a week, and subsequently the 10 birds with the fastest erections and semen ejaculation, and well-developed foam glands were selected for further experiments.

In the second stage of the experiment semen was collected by stimulation of the male with a teaser female, as elaborated by Chełmońska et al. (12) for Muscovy drakes. The procedure adopted for quails began by taking the male from the cage and placing it on the left hand with the bird's breast in the palm of the hand; wings and legs were held up. The foam was removed from the cloacal gland by delicate squeezing of the gland with the thumb of the left hand and the forefinger of the right hand. After foam removal, the male was put back in the cage and then a female was placed in the cage. In order to prevent unintentional copulation the palm of the hand was placed under the male's cloaca (Figure 1). At the moment of intense male excitation, manifested by the characteristic arching of the back and spreading of the wings, the male was quickly taken from the cage. Immediately thereafter, the lateral walls of the cloaca were pressed, the copulatory organ was everted, and dense, viscous semen appeared. In all, 12 semen collections were performed for each male.

Females used for male stimulation were sexually mature (in a laying phase) and characterised by a quiet temperament; they displayed the tolerance reflex, enabling males to ascend on them quickly.

Semen was collected twice a week from each male. Extruded semen was collected from the everted copulatory organ with the use of a small glass collector fitted, with rubber tubing and a mouthpiece, into a small calibrated tube enabling measurement of ejaculate volume exact to 10 µl (Figure 2). For proper semen collection, 2 operators were necessary.

In freshly collected semen the following parameters were evaluated: ejaculate volume, colour, density, blood or faecal contamination, and concentration and morphology of spermatozoa.

The spermatozoa concentration was measured with a haemocytometer.

The morphology of spermatozoa was studied in nigrosin-eosin smears (13) under a light microscope (Jenaval, Carl Zeiss; 1250× magnification). In every smear, 300 spermatozoa were categorised into 7 classes; among them, 6 classes of live unstained spermatozoa regarded as total live spermatozoa, i.e. morphologically normal, macrocephalic, bent-neck, midpiece deformed spermatozoa, spermatids (immature cells), spermatozoa with other deformities, as well as dead spermatozoa stained by eosin were examined (14).

Additionally, the rate and type of male reactions to semen collection were noted in the second stage of the experiment.

In order to provide a reliable evaluation of semen quality and comparison between males, the semen quality factor (SQF), calculated according to the following pattern was used:

Figure 1. Palm of the hand placed under male cloaca to prevent undesirable copulation.

Figure 2. Calibrated quail semen collector.

$$SQF = \frac{\text{sperm concentration } (n \times 10^6\ ml^{-1}) \times \text{ejaculate volume (ml)} \times \text{live normal spermatozoa (\%)}}{100\%}$$

Differences in semen quality resulting from individual male characteristics were analysed by ANOVA and Duncan's multiple range tests (SAS system, General Linear Models Procedure).

Results

Male Reactions to the Semen Collection Procedure

On the basis of male response to semen collection, 4 types of reactions were distinguished:

Type I: Male demonstrated intense excitation and responded immediately after placing the female into his cage by ascending onto the female's back and attempting to copulate.

Type II: Male produced valuable ejaculates, but a slightly longer stimulation was required than in type I (about 30 s),

Type III: Male required longer stimulation (more than 60 s) and produced a small volume of semen, usually mixed with foam or contaminated by blood or faecal matter.

Type IV: Male did not react to the presence of the female.

Only type I and II reactions (Figure 3) were recognised as positive and satisfactory from the artificial insemination viewpoint; type III and IV reactions were considered negative.

It should be stressed that during subsequent semen collections different reactions were also observed in the same male.

Figure 3. Quail semen collection.

Characteristics of the Collected Semen

Quail semen, in density and appearance, resembled yellow-tinted, condensed milk. Sporadically, ejaculates produced by very excitable males were contaminated by blood. Among the 10 males subjected to semen collection, 3 were excluded from further calculations because of an insufficient number of desired reactions (6-7 positive out of 12 collections).

Spermatozoa concentration per ejaculate collected during the entire experimental period and from all males averaged 693 $\times$ 10^6 ml^{-1} (range: 592-812 $\times$ 10^6 ml^{-1}). Significant differences ($P \leq 0.05$) in sperm concentration were observed only for 2 males (592 vs. 812 $\times$ 10^6 ml^{-1}) (Table).

Similarly, ejaculate volume differed with respect to individuals (Table). The average volume of all ejaculates collected during the experimental period was 27 µl (range for individuals: 20-35 µl). The total amount of live spermatozoa varied from 83.0% to 99.3%. Only one male produced ejaculates with significantly ($P \leq 0.05$) less spermatozoa (87.6%, on average). The quantity of live morphologically normal cells varied from 70.0% to 95.0% (average: 83.4% for all collected ejaculates) (Table). Differences in the number of live normal spermatozoa observed for individual males were significant ($P \leq 0.05$) only in the case of one male.

A small amount of macrocephalic spermatozoa (1.0%-8.6% in individual ejaculates), bent-neck cells (0.0%-4.6%), and midpiece deformed spermatozoa (0.0%-2.6%) were recorded (Table).

The SQF comprises 3 semen traits and is the most important parameter from the viewpoint of semen usability for artificial insemination and spermatozoa fertilizing ability; it adequately characterises the ejaculate value and depicts differences among the males. In the presented experiment the differences among males were not significant, although average SQF value varied from 11.4 to 19.2 (however, for individual ejaculates the range was 3.6-37.0). Usually, males that produced ejaculates of high volume had small spermatozoa concentrations.

Table. Characteristics of the fresh semen collected individually from Japanese quail by female stimulation of the male (means ± SD).

No. of ♂	Collections [n]	Sperm concentration [$\times$ 10^6 ml^{-1}]	Ejaculate volume [ml]	Sperm quality factor[1]	Classes of spermatozoa [%]					
					Live in total	Live normal	Macro-cephalic	Bent-neck	Midpiece changes	Other deformities
1	11	801.0 ± 222.83	0.027 ± 0.011	19.2 ± 10.57	94.6^{a} ± 2.02	84.8^{a} ± 4.38	2.4 ± 1.52	1.6 ± 0.85	0.6^{b} ± 0.75	5.2 ± 2.47
2	10	632.2 ± 120.62	0.035^{a*} ± 0.013	18.59 ± 9.10	94.1^{a} ± 3.74	82.9^{a} ± 4.30	3.0 ± 1.85	2.0^{a} ± 1.17	1.2^{a} ± 0.71	5.0 ± 2.88
3	10	812.2^{a} ± 204.01	0.026 ± 0.011	18.3 ± 8.16	95.2^{a} ± 2.13	86.4^{a} ± 3.99	2.9 ± 2.47	2.0^{a} ± 0.76	0.7 ± 0.24	3.2 ± 2.74
4	10	665.5 ± 153.06	0.033^{a} ± 0.013	17.8 ± 6.72	92.5^{a} ± 3.16	82.8^{a} ± 6.10	2.6 ± 1.4	1.4 ± 0.91	0.9 ± 0.79	4.8 ± 3.53
5	10	592.0^{b} ± 119.05	0.025 ± 0.010	12.3 ± 6.10	94.2^{a} ± 3.88	84.5^{a} ± 6.59	3.0 ± 2.08	1.1^{b} ± 0.56	1.2^{a} ± 0.50	4.4 ± 3.29
6	10	709.0 ± 182.48	0.020^{b} ± 0.011	11.5 ± 9.00	94.8^{a} ± 3.33	84.8^{a} ± 3.91	2.4 ± 2.20	2.1^{a} ± 1.16	1.2^{a} ± 0.60	4.3 ± 1.21
7	10	643.3 ± 348.64	0.023 ± 0.015	11.4 ± 10.13	87.6^{b} ± 4.26	77.1^{b} ± 6.23	3.3 ± 2.12	0.8^{b} ± 0.62	0.5^{b} ± 0.52	5.9 ± 2.83
Means for all males		693.0±210.08	0.027 ± 0.012	15.6 ± 8.95	93.4 ± 3.98	83.4 ± 5.63	2.8 ± 1.90	1.6 ± 0.97	0.9 ± 0.64	4.7 ± 2.76

*[a,b] Means within columns followed by different superscripts differ significantly ($P \leq 0.05$).

[1] SQF: spermatozoa concentration (n $\times$ 10^6 ml^{-1}) $\times$ ejaculate volume (ml) $\times$ live morphologically normal spermatozoa (%)/100%.

Discussion

The obtained results suggest that, in artificial insemination of Japanese quail, male stimulation by a female teaser is an effective method of semen collection, resulting in valuable ejaculates from most of the treated males. This method is also less stressful than the dorso-abdominal massage method normally used for semen collection from different bird species. Our further experiments on quail insemination indicated that well selected and trained males only sporadically do not respond to stimulation by a female teaser. Of 720 semen collections, as many as 715 ejaculates were obtained: a 99.3% positive reaction rate (15). Moreover, quail semen donors that were accustomed to the semen collectors displayed the fastest reactions and greatest inclination to ejaculate. Right after the removal of the proctodeal gland foam, followed by placing the male back in the cage, they ejaculated semen spontaneously. The effectiveness of this method is probably the result of engendering the male's natural sexual reflex. When male stimulation by a female teaser was used, male sexual incitement, erection, and ejaculation occurred within a few seconds, while during stimulation with massage about 1 min was necessary (10). However, it has to be pointed out that in both methods success depends on the selection of appropriate males and how accustomed they are to the procedures. Moreover, the presence of some unknown people and objects is very stressful for birds and negatively affects the male reaction, and consequently the quality of semen. When the massage method is used, the collectors participating in the procedure should be patient and calm. When male stimulation by a female teaser is performed, the moment when the male should be separated from the female can be difficult to notice. Therefore, knowledge of the bird's behaviour and experience in pressing the lateral wall of the cloaca are very important in overcoming such difficulties.

In the present experiment 2 weeks were necessary for the males to become accustomed to the semen collection procedure, which is congruent with the experiment by Chełmońska and Gałuszka (16) carried out on drakes, and that by Michel (17) on roosters.

In order to assure quick and a proper semen collection, males must be selected carefully on the basis of cloacal gland size, the rate of response to the presence of a female, and, as stressed by many authors (6,18,19), they should be kept individually. Male quails chosen for semen collection should be characterised by a well-developed proctodeal gland intensively filled with foam of dense consistency. Size and colour of this gland is closely correlated with male hormonal activity. Furthermore, placement of a cage with females in the same room as individual cages with males, in order to intensify the male sexual impulse, is highly advisable.

The quantity and quality of semen is dependent on individual male characteristics; however, differences observed among quails were not as significant as in other bird species (20). The volume of ejaculate collected from Japanese quail is one of the smallest among all poultry species. Ejaculates of similar volume (10 µl) were collected by Wentworth and Mellen (5), Baumgartner (21) (10-20 µl), and Tarasewicz et al. (9) (5-20 µl). Comparative data suggest that stimulation of the male by a female teaser creates better conditions for collecting semen of greater volume.

Spermatozoa concentration of quail semen collected in the present experiment was higher than that obtained by Fujihara and Koga (22) (42.62×10^6 ml^{-1}), Baumgartner (21) ($52\text{-}59 \times 10^6$ ml^{-1}), Bunaciu et al. (23) ($220\text{-}330 \times 10^6$ ml^{-1}), Buxton and Orcutt (24) (469.5×10^6 ml^{-1}), and Tarasewicz et al. (9) ($120\text{-}312 \times 10^6$ ml^{-1}), but was not as high as in the experiment by Wenworth and Mellen (5) (1200×10^6 ml^{-1}). However, our latest experiments (25) indicate that with proper male selection, in accordance with the rules described above, spermatozoa concentration can be $2240\text{-}2640 \times 10^6$ ml^{-1}.

The number of all deformed spermatozoa amounted for 16.0% and was higher than in the experiment by Bunaciu et al. (23) (4.05-6.35%), and Brożek and Knothe (26) (0.14-0.34%); however, the latter authors indicated deformations only within the sperm head.

SQF used for evaluating individual quail semen seems to be a good predictor of semen fertilising ability. Moreover, it can be performed in a simple farm laboratory equipped with a light microscope and a hot plate, and the evaluation repeated by the same person prevents operator-dependent errors. Although some of the evaluated quails differed significantly ($P \leq 0.05$) in ejaculate volume, sperm concentration, and number of live morphologically normal cells, their SQFs were similar, since usually smaller semen volume was compensated for by higher spermatozoa concentration. The correlation between ejaculate volume and sperm concentration, calculated for gander semen, was negative and

significant. The values of SQF of gander semen vary within a wider range (7.7 to 53.2) (19).

The results of the present experiment revealed that male stimulation by a female teaser, which results in ejaculates of large volume, and high spermatozoa concentration and number of live morphologically normal cells, can be highly recommended for use in future studies on quail reproductive biology.

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