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Brief report

Successful semen collection from Capercaillie (*Tetrao urogallus* L.) kept in an aviary system

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We tested the feasibility of obtaining ejaculates from Capercaillie (*Tetrao urogallus* L.) kept under aviary conditions. A total of nine individuals in their first ($n = 4$), fifth ($n = 3$) or sixth ($n = 2$) reproductive season were raised in floor pens in the absence (1-year old males) or presence (5- and 6-years old males) of contemporary females and attempted to collect semen using the massage technique. Successful ejaculations were more frequently obtained in older (90%) than in young males (50%). The following semen characteristics of ejaculates were observed for young vs. old males: mean volume 25µl vs. 41µl; sperm concentration $470 \times 10^6 ml^{-1}$ vs. $1{,}133 \times 10^6 ml^{-1}$; sperm viability (eosin-nigrosin test) 92.7% vs. 94.6%; morphologically normal sperm 66.5% vs. 73.2%. Semen quality, expressed as the Sperm Quality Factor, reached 10.5 in young and 33.8 in older males. These observations confirm the feasibility of performing successful semen collection in captive Capercaillie, a pre-requisite for the development of artificial insemination for the purpose of gene banking and to optimize the reproductive performance in this endangered species. Interestingly, one-year-old Capercaillie can produce semen, although the quantity and quality are lower than in older males.

1. Introduction

The European population of Capercaillie (*Tetrao urogallus* L.) (Russia excluded: no statistics available) is currently limited to about 580,000 individuals of which 500,000 occupy Scandinavia. Over the past half century, this population has markedly decreased in most European countries, including Poland. This decline accompanied, or was a consequence of, a rapid reduction of their natural habitat. As in many other feral avian species, the causal factors known to have a negative impact on Capercaillie populations include unfavorable forest management, human-caused perturbation of their habitats, and increased density of natural predators. In Poland, the population of Capercaillie is presently limited to 480–650 individuals living in four isolated sub-populations. In order to counteract this quasi-disappearance, the Polish authorities have established two aviary breeding centers located in the Leżajsk and Wisła Forest Districts (Rutkowski *et al*. 2005, Dziedzic *et al*. 2006).

Despite the reduction in the genetic variability, caused by isolation of the four local populations, the Polish Capercaillie populations have apparently retained a substantial level of microsatellite polymorphism (Rutkowski *et al.* 2005). Such observations prompted us to test the feasibility of obtaining ejaculates from captive individuals, a prerequisite to establish artificial insemination programs tailored to this species and to develop gene banks facilitating international exchange of biological material to preserve genetic diversity and reduce the risks of disease transfer (Saint Jalme *et al*. 2003).

The dorso-abdominal massage technique, first described by Burrows and Quinn (1937) to collect semen in the chicken, has since been successfully used on a number of poultry and feral species (Blanco *et al*. 2009, Łukaszewicz *et al*. 2004). Successful semen collection constitutes a common baseline for artificial insemination (AI) technology as well as for basic or applied research in male fertility. In all species, the reproductive success of an AI program depends on semen quality which itself depends on the ability to obtain sperm in adequate quantity and quality from males (Montovani *et al*. 1993, Saint Jalme *et al*. 1994, 2003, Zhang 2006).

In several lek mating systems, centrally-located males have a higher mating success than peripheral individuals (Tsuji *et al*. 2000). In addition, dominant males occupying centrally-located territories have significantly larger testes, higher testosterone levels and better semen quality than have juveniles (Mjelstad 1991, Alatalo *et al*. 1996,Tsuji *et al*. 2000,). Using a path analysis, Garamszegi *et al*. (2005) showed that relative testis size primarily evolved in association with intense sperm competition and thus high sperm production. According to Tsuji *et al.* (1992), a female may use the body mass of a male as a further cue to assess the probability that sperm from a potential mate will fertilize all ova. However, the observations by Mjelstad (1991) suggest that the quality of semen may also, at least partly, depend on the intensity of displays. Capercaillie coming from aviary breeding and those from natural habitats show remarkable behavioral similarities (Dziedzic *et al*. 2006). Despite the fact that hens had access to all males in that study, they tended to spend most time with the dominant individuals which were thus the main candidates for reproduction. These results suggest that captive birds have not necessarily lost their natural instincts.

In the present study, we tested the feasibility of obtaining ejaculates from Capercaillie kept under aviary conditions and, whenever successful, to assess the quantitative and qualitative characteristics of ejaculates collected from young and dominate males for further applications in artificial insemination and gene banking.

2. Material and methods

2.1. Experimental birds

The experiment was carried out at the Capercaillie Aviary Breeding Center of the Wisła Forestry Management, Poland, in April/May, i.e., one month after the onset of the reproductive season in the geographical region. A total of nine males having never been trained for semen collection were used, from which four were in their first (designated as young males), three were in their fifth and two were in their sixth reproductive season (these latter two classes were designated as older males). Young males were kept individually in floor pens (without females, but in vocal and visual contact with these), while older males, with an exception of one six-years-old male (male number 5), were kept isolated from other males but were provided a constant presence of 2–3 females. These males were allocated a separate roofed space of 7.0 m × 4.0 m × 3.0 m (length, width and height) adjoining female rooms in their longer dimensions through calibrated holes (17 cm in diameter) opened in the lateral walls. This allowed females, but not males, unlimited pass to the male pen.

2.2. Semen collection, and quantitative assessment of ejaculates

Semen collection was performed twice per day, in the morning and in the afternoon, on Monday and Wednesday, using the dorso-abdominal massage technique modified from Burrows and Quinn (1937). Sperm concentration was estimated by

Table 1. Characteristics of freshly collected ejaculates from Capercaillie in their first (young males), and fifth and sixth (old males) reproductive season. * = Males kept with females; ** = males kept alone; # = Males from which transparent fluid rather than semen was collected. SQF = Semen Quality Factor (see text).

Male number	Reproductive season	Successful collections	Sperm concentration [$\times 10^6$ ml^{-1}]	Ejaculate volume [ml]	SQF	Sperm morphology (%)						
						Live, total	Normal	Bulb-head	Crooked neck	Altered Mid-piece	Other deformities	Dead
1*	6	2	1,900.0	0.055	63.4	94.0	66.7	18.7	3.3	3.4	1.9	6.0
2*	7	2	1,270.0	0.035	31.7	95.3	77.8	8.7	1.8	5.7	1.3	4.7
3*	6	2	705.0	0.060	22.6	98.2	74.3	11.2	2.2	10.2	0.3	1.8
4*	6	1#	350.0	0.020#	4.8	85.7	65.3	14.0	2.7	3.7	0.0	14.3
5**	7	2	1,440.0	0.040	46.9	95.3	82.0	7.6	2.7	2.3	0.7	4.7
Old males, mean ± SD	–	9	1,133.0 ± 611.3	0.041 ± 0.015	33.8 ± 22.4	94.6 ± 3.9	73.2 ± 6.9	12.7 ± 6.2	2.5 ± 1.0	4.9 ± 3.5	1.2 ± 1.1	5.4 ± 3.9
6**	1	0	–	–	–	–	–	–	–	–	–	–
7**	1	1	1,220.0	0.055	29.9	87.0	45.3	37.4	0.3	3.0	1.0	13.0
8**	1	2#	60.0	0.010#	0.4	94.2	74.2	11.8	3.7	3.8	0.7	5.8
9**	1	1#	150.0	0.010#	1.0	92.5	68.5	13.4	2.0	8.3	0.3	7.5
Young males, mean ± SD	–	4	470.0 ± 0.52	0.025 ± 0.022	10.5 ± 12.6	92.7 ± 4.2	66.5 ± 11.0	17.0 ± 10.8	3.3 ± 2.5	5.5 ± 3.3	0.5 ± 0.4	7.3 ± 4.2
All males, mean ± SD	–	13	905.1 ± 601.1	0.038 ± 0.019	25.5 ± 20.7	93.3 ± 4.4	71.0 ± 8.8	13.4 ± 8.6	2.8 ± 1.6	5.1 ± 3.3	1.0 ± 0.9	6.7 ± 4.0

hemocytometry using a 3% eosin-NaCl solution (v/V) and Thoma-type grids. Sperm integrity and morphology was examined in nigrosin-eosin smears (300 sperm/slide examined) and assessed at 1,250 × magnification under light microscopy (Nikon Eclipse E100). Sperm were morphologically categorized into six classes as described by Gwara *et al.* (2004). Results were expressed as percent sperm bearing to 1 of the 6 pre-defined categories (300 cells = 100%).

In order to provide a reliable assessment of the overall "semen quality" for further comparisons between males, Semen Quality Factor (SQF) was calculated for each ejaculate (Łukaszewicz & Kruszyński 2003). This synthetic parameter comprises three semen traits generally considered as the most important from the viewpoint of sperm fertilizing ability. It characterizes a "global" ejaculate value and can also be used to compare male fertilizing potential between individuals or between groups of males. This SQF can be calculated as follows: sperm concentration ($\times 10^6$ ml^{-1}) × ejaculate volume (ml) × normal spermatozoa (%) /100%.

2.3. Statistical analysis

Depending on the success of semen collection, the results of single or average data of multiple collections were considered. The data of the young and older male semen quality were averaged and SD was calculated using STATISTICA version 7.1 (StatSoft Inc.).

3. Results

When successful, responses to dorso-abdominal massage were observed within two minutes irrespective of the age of a male. A positive response to massage was observed for 8 out of the 9 individuals tested, 5 of them being old and 3 being young. On average, 90% of ejaculations were successful for old males, whereas the percentage was about 50 for young males. Ejaculate volumes were smaller for young than for old males (25µl vs. 41µl; Table 1). Sperm concentration in ejaculates collected from young males averaged 470 × 10^6ml^{-1} compared with 1,133 × 10^6ml^{-1} in old males. The proportion of live sperm in ejaculates was similar for both age groups (averages 92.7% for young vs. 94.6% for old males). However, the mean percentage of motile and morphologically normal sperm of young males represented 66.5% of total ejaculated, compared with 73.2% in ejaculates collected from old males. The most frequently encountered abnormalities for both groups were bulb-head deformations (Table 1). Sperm characteristics were used to establish both individual and intra-group SQF. The SQF were higher for old (33.8 ± 22.4 SD), than for young males (10.5 ± 12.6 SD).

4. Discussion

This study demonstrates the feasibility of obtaining ejaculates from Capercaillie using the dorso-abdominal massage method. As many as 72% of the Capercaillie subjected to this technique responded positively at their first massage either by excreting transparent fluid, including some viable sperm, or by ejaculating semen with varying sperm concentrations. According to Montovani *et al.* (1993), only 10% of male Common Pheasants (*Phasianus colchicus mongolicus*) ejaculated when subjected to their first dorso-abdominal massage. Durrant *et al.* (1995), working with Himalayan Monal (*Lophophorus impejanus*), obtained on average 53% successful ejaculations at the first reproductive season, but this percentage reached 94% and 93% during the third and fourth reproductive seasons, respectively.

In the present study, Capercaillie expressed a positive effect of age on successful stimulations. Thus, semen collections performed on first-year males resulted in an ejaculation only in about 50% of the massage stimulations, whereas older males responded positively in nearly 90% of the stimulations. Such differences in the frequency of achieved ejaculations may result from an increased susceptibility of young males to the massage procedure, including handling preparation. In pheasants, Saint Jalme *et al.* (2003) compared different species reared under similar captive conditions, and observed highly variable responses to massage stimulation between individuals of different genetic origin. In sexually mature chickens, the peak frequency of positive responses to the massage technique can only be reached after at least two weeks of repeated training (at least twice a week; J.-P. Brillard, pers. comm.).

In our study, semen quality varied not only between individuals but also between the two age groups, an indication that young males may not have reached full reproductive efficacy, as shown for both domesticated and feral avian species (Mjelstad 1991, Chełmońska *et al.* 2008). Here, young Capercaillie were able to produce viable sperm, but the overall quality of their ejaculates was generally lower than in older males. This observation supports Mjelstad (1991) in that sub-adult (second-year) Capercaillie are not necessarily physically ready to efficiently mate with females. Indeed, the frequency of individual displays is positively correlated with semen quality traits ($r_s = 0.94$; present data). This interaction between behavioral and physiological traits has also been described in other grouse species. Tsuji *et al.* (2000) reported that adult Sharp-tailed Grouse (*Tympanuchus phasianellus*) occupying centrally-located territories on the tooting grounds have significantly larger testes than have juvenile and peripheral individuals. In poultry species, the body size of juvenile males can be taken as a general, albeit imprecise, indicator of testicular development and of ejaculate volume, as shown for the Guinea fowl (Brillard & de Reviers 1985). Thus, a female may use body size of a male as a cue to assess its reproductive potential for the fertilization of a maximum number of ova (Tsuji *et al.* 1992).

In the present study, ejaculate volumes appeared similar to those obtained from tragopans (*Tragopan* spp.; extremes 33.0–43.3µl; Durrant *et al.* 1995, Saint Jalme *et al.* 2003, Zhang 2006), while sperm concentrations were comparable to those of *Pucratia macrolopha* (1,200 × $10^6 ml^{-1}$, Saint Jalme *et al.* 2003) and *Tragopan temminckii* (1,280 × $10^6 ml^{-1}$, Durrant *et al.* 1995). However, the concentrations observed here were lower than in other pheasant species (from 2,300 to 6,600 × $10^6 ml^{-1}$; Montovani *et al.* 1993, Saint Jalme *et al.* 2003, Zhang 2006). The frequency of semen collection applied in our study (two days of semen-collection events/week and two collection events per day) may have negatively affected ejaculate volumes. In the Cabot's Tragopan (*Tragopan caboti*), Zhang (2006) observed a marked decrease in concentration of semen collected once a day rather than every 3–6 days (400 × 10^6ml^{-1} compared to 1,930–2,070 × 10^6ml^{-1}, respectively). Similar observations had earlier been described in the Houbara Bustard (*Chlamydotis undulata*; Saint Jalme *et al*. 1994) but in some other species, such as the Wild Turkey (*Meleagris gallopavo*), increased frequency of semen collection results in a decreased ejaculate volume rather than decreased sperm concentration (Noirault & Brillard 1999). This pattern indeed justifies the need to perform species-specific research to obtain precise ejaculate parameters.

The fertilizing ability of spermatozoa is at first dependent on the motility and morphology of spermatozoa. In this study, the percentage of motile and morphologically normal sperm ranged from 66.5% in young to 73.2% in older males, which is similar to observations for *Tragopan temminicki* (70%) and *T. caboti* (65.9–68.9%) (Saint Jalme *et al*. 2003) and for *Phasianus colchicus mongolicus* (Marzoni *et al*. 2000). In poultry species, the percentage of motile and morphologically normal sperm reached 64% in turkey and 69% in Guinea fowl (Blesbois *et al*. 2005). However, despite intense selection over decades, inter-breeding variability is high in *Gallus domesticus* (range from 56.4% to 94.0%; Siudzińska & Łukaszewicz 2008, Łukaszewicz *et al*. 2008). However, sperm abnormalities observed in Capercaillie semen were similar in type to those described in the Cabot's Tragopan (Zhang 2006). The percentage of abnormalities in our study was 22.0%, a value higher than previously described, e.g., in the rooster (15.3%; Siudzińska & Łukaszewicz 2008) and in Cabot's Tragopan (11.7%; Zhang 2006).

In the present study, SQF values were lower than those calculated for chicken semen (0.4–63.4 vs. 75.9–132.2; present data and Siudzińska & Łukaszewicz 2008). Major differences in SQF values have previously been reported between Greylag Goose (*Anser anser* L.) and the domestic White Koluda Goose (1.06–1.64 vs. 19.26–56.52, respectively; Łukaszewicz *et al*. 2004; Łukaszewicz & Kruszyński 2003). Here, the differences in SQF between young (10.5) and old (33.8) Capercaillie were mainly caused by differences in sperm concentration and, to a lower degree, by ejaculate volume. Comparisons of fertility between female Capercaillie subjected to artificial insemination with semen from young and old males would be required to verify if such differences in SQF are followed by correlated differences in female and egg fertility.

The present experiment opens interesting premises for the development of AI technology in Capercaillie kept in aviaries. Based on the present observations, the frequency of positive responses to massage stimulation as well as the overall quality of ejaculates appear sufficient to ensure a rapid development of this technique in purposes such as restoring populations of Capercaillie by means of assisted reproduction.

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Onnistunut siemennesteen keruu tarhakasvatetuilla metsoilla

Selvitimme, onnistuuko siemennesteen talteenotto tarhakasvatetuilta metsoilta (*Tetrao urogallus*). Kaikkiaan yhdeksää metsokukkoa (neljä yksivuotiasta, kolme viisi- ja kaksi kuusivuotiasta) kasvatettiin lattiakasvattamossa naaraiden poissa- (yksivuotiaat kukot) ja läsnäollessa (vanhemmat kukot). Siemennestettä pyrittiin saamaan hierontatekniikalla. Siemensyöksy oli yleisempää vanhoilla (90 % yrityksistä) kuin nuorilla kukoilla (50 % yrityksistä).

Seuraavat siemennesteen piirteet havaittiin nuorilla ja vanhoilla kukoilla: keskitilavuus 25 vs. 41 µl; siittiöpitoisuus 470 × 106 vs. 1 133 × 10^6 ml^{-1}; siittiöiden elinkyky (eosiini-nigrosiini -testi) 92,7 vs. 94,6 %; ja morfologisesti normaali siemenneste 66,5 vs. 73,2 %. Siemennesteen laatu, arvioituna spermanlaatufaktorilla, oli 10,5 nuorilla ja 33,8 vanhoilla kukoilla. Havainnot varmistavat, että siemennestettä on mahdollista menestyksellä tuottaa tarhaoloissa pidetyillä metsoilla, mikä on edellytys keinohedelmöitykselle, geenipankkitoiminnalle ja tämän uhanalaisen lajin lisääntymisen varmistamiselle. Huomionarvoista on, että jo yksivuotiaat kukot kykenevät spermantuotantoon, vaikkakin siemennesteen määrä ja laatu ovat alhaisempia kuin vanhoilla kukoilla.

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