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Brief report

Sperm characteristics of male hybrid from a cross between male Black Grouse *Tetrao tetrix* and female Capercaillie *T. urogallus*

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1. Introduction

Crosses between Black Grouse *Tetrao tetrix* male and Capercaillie *T. urogallus* female are the most common grouse hybrids (Höglund and Porket 1989, McCarthy 2006). Such hybrids were recorded in Central Europe for the first time in 1744 (Johnsgard 1983) but likely must have been known earlier since ranges of these species overlap. Hybrids especially appear when a distorted sex ratio occurs in favor of Capercaillie hens. Porket (1990) indicated such a distortion in Central Europe did occur at the end of 19th century and the beginning of 20th century when a large number of Capercaillie males were killed as a result of prestige-motivated spring hunting, and during the 1950s and 1960s. In the latter, anthropogenic factors are to blame for creating unfavorable environmental conditions for the survival of male Capercaillie chicks.

Unlike females, some male hybrids (F_1 generation) are assumed to be fertile and produce offspring (F_2 generation) with females of both Capercaillie and Black Grouse (Porkert *et al.* 1998). Lekking behavior of hybrids has been reported (Porkert *et al.* 1997) and backcrossings have been obtained in captivity (Höglund and Porket 1989, Porkert *et al.* 1997, 1998). Variable fertility of hybrids has been described and attributed to variation in sperm quality (Porkert *et al.* 1998).

To our knowledge, the only available information concerning semen of male hybrid from a cross between male Black Grouse and female Capercaillie is reported by Höglund and Porket (1989) as "Hybrid 1 cocks can be fertile (living sperm, though less motile than capercaillie ♂)". Unfortunately, we could not find information concerning methods and data supporting this statement, due to a lack of reference information concerning the hybrid semen. Hence, the objective of the present study was to obtain basic data concerning semen characteristics of male hybrid from a cross between Black Grouse and female Capercaillie. Because male hybrids from a cross between Black Grouse and female Capercaillie are extremely rare, we were able to use only one hybrid in this study.

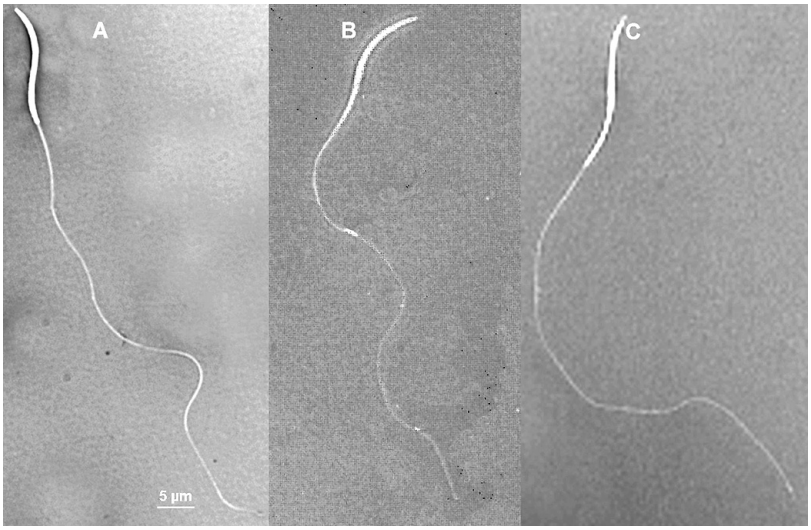

Fig. 1. Light microscopy of normal spermatozoa of Capercaillie (A), Black Grouse (B) and male hybrid from a cross between Black Grouse and female Capercaillie (C).

2. Material and methods

A three-year-old hybrid was bred after crossing of a *T. tetrix* male and a *T. urogallus* female in the Kadzidłowo Wildlife Park, Poland. The Capercaillie female was three years old and the Black Grouse males were two or three years old. Sperm was collected with the use of the dorso-abdominal massage technique (Burrows and Quinn 1937), three times during the reproductive season (April–May), at a week interval. The hybrid exhibited lekking behavior during this period. Two semen samples were obtained from the hybrid, two samples from Capercaillie, and four from different male grouses.

Morphology of the spermatozoa (n = 300 cells per slide) was examined in nigrosin-eosin smears and evaluated at 1,000× under a light microscope (Nikon Eclipse E 100). Spermatozoa were categorized using similar classification as described previously (Łukaszewicz *et al.* 2004). Nine morphological categories were distinguished: (1) normal, (2) swollen, (3) defective acrosome, (4) bent neck, (5) looped heads, (6) defective midpiece, (7) spermatids, (8) dead cells, (9) other defects (not included in the previous categories such as coiled tails, lack of tail, and deformed head without tail). The results of the morphological examination were expressed as the percentage of particular categories of spermatozoa (300 cells = 100%). Sperm concentration of the freshly collected semen was calculated in a hemocytometer with the use of eosin-3% NaCl solution and Thoma-Zeiss chamber.

Morphometry of the spermatozoa was assessed on the basis of histological smears vital-stained by a nigrosin-eosin complex. For every species 100 spermatozoa were measured under a light microscope at 1250× magnification with the use of a Nikon Eclipse 50i microscope equipped with a Lucia G computer-assisted program for picture analysis. The length of sperm head, midpiece, tail and entire spermatozoa was measured.

Motile sperm were video recorded using a microscope with a 10× negative phase lens and a Sony CCD black-and-white video camera (SPT-M108CE). The video recordings were analyzed using a Hobson Sperm Tracker (Hobson Vision Ltd, Baslow, UK). MicroCell 20 mm, 2-chamber slides (Conception Technologies, San Diego, California, USA), were mounted on a heated stage (38 °C). Conditions of sperm dilutions, recording and Hobson Tracker settings followed those described by Kotłowska *et al.* (2007). However, the semen predilution step was omitted due to a low concentration of spermatozoa and TES in the activation buffer was replaced by Tris. Motility parameters used in this study included the following: curvilinear velocity (VCL); straight-line velocity (VSL), amplitude of lateral head displacement (ALH), linearity (LIN), and beat-cross frequency (BCF). Since only one specimen of hybrid was available, only descriptive statistics are shown. Data are presented as means ± SD.

Table 1. Concentration and morphology of spermatozoa from a cross between Black Grouse (Hybrid) and female Capercaillie, and pure Black Grouse and Capercaillie. Morphological categories: (1) normal, (2) swollen, (3) defective acrosome, (4) bent neck, (5) looped heads, (6) defective midpiece, (7) spermatids, (8) other defects, (9) dead cells.

Taxon	Concentr.	Morphological category (%)								
	(× 10^9 ml–1)	1	2	3	4	5	6	7	8	9
Hybrid (*n* = 1)	0.610	29.0	14.6	1.0	3.0	4.0	3.3	2.4	15.9	26.8
Black Grouse (*n* = 4)	1.260	59.2	18.9	0.3	3.7	0.0	5.6	0.0	0.0	12.3
Capercaillie (*n* = 1)	1.645	53.0	14.4	0.0	2.5	0.0	6.6	0.3	3.2	20.0

3. Results

For typical morphology of spermatozoa of *T. urogallus*, *T. tetrix*, and male hybrid from a cross between Black Grouse and female Capercaillie, see Fig. 1. Sperm concentration of the hybrid semen was about half of that of Capercaillie and Black Grouse (Table 1). The semen of the hybrid contained the lowest percentage of normal spermatozoa and, consequently, the highest percentage of spermatozoa with abnormal morphology. The average length of spermatozoa was within the range of 70–80 µm for all three taxa, and the spermatozoa of the hybrid had the highest length of the head (Table 2).

All morphometric parameters for the hybrid spermatozoa had higher variability (SD) as compared to Capercaillie and Black Grouse. Percentage of sperm motility of the semen of the hybrid was considerably lower as compared to that of Capercaillie and Black Grouse (Table 3). However, the velocity of sperm movement seemed to be similar for all three taxa.

4. Discussion

Our results provide descriptive information concerning characteristics of male hybrid from a cross between Black Grouse and female Capercaillie for the first time. Viable spermatozoa were identified with the capability to move with movement characteristics similar to that of the parental species. Gross morphology of the viable spermatozoa of the hybrid did not differ from that of Capercaillie and Black Grouse. However, semen quality was

Table 2. Morphometry of spermatozoa of a male hybrid from a cross between Black Grouse and female Capercaillie, and pure Black Grouse and Capercaillie. The values represent mean ± SD µm.

Taxon	Head	Midpiece	Flagella	Total
Hybrid (*n* = 1)	13.98 ± 2.62	6.72 ± 0.98	65.52 ± 3.83	86.22 ± 4.89
Black Grouse (*n* = 4)	10.71 ± 0.71	6.73 ± 0.79	64.12 ± 2.92	81.56 ± 2.97
Capercaillie (*n* = 1)	9.73 ± 0.55	5.92 ± 0.81	57.62 ± 2.97	73.27 ± 3.03

Table 3. Motility parameters of semen of a male hybrid from a cross between Black Grouse and female Capercaillie, and pure Black Grouse and Capercaillie. Motility = % of motile sperm; VCL and VSL are in µm s^{-1}; BCF is in Hz; and ALH is in µm.

Taxon	CASA parameter				
	Motility	VCL	VSL	BCF	ALH
Hybrid (*n* = 1)	46.0	102.8	65.5	9.1	10.0
Black Grouse (*n* = 4)	76.8	104.5	64.1	8.2	10.4
Capercaillie (*n* = 1)	79.5	103.9	57.6	6.7	12.7

lower in the hybrid than in Capercaillie and Black Grouse.

Although no statistical comparisons could be performed, it is worth mentioning that the hybrid semen was characterized by lower values of quality characteristics, such as sperm concentration, percentage of spermatozoa with normal morphology and percentage of sperm motility. Interestingly, the hybrid was characterized by the largest size of the sperm head. Barna and Wishart (2003) described spermatozoa with elongated nuclei in Guinea Fowl (*Numidia meleagris*). These authors linked the presence of large sperm nuclei with excess nuclear DNA including polyploidy. It has to be established if this phenomenon is present in the semen of hybrid *T. tetrix* male x *T. urogallus* female. Lower values of semen quality parameters and their high variability correspond to the low fertility of hybrids reported earlier (Porkert *et al.* 1998).

Although the percentage of sperm motility was low for the hybrid, which agrees with earlier findings by Höglund and Porket (1989), their velocity parameters were similar to parent species. In other words, when spermatozoa of the hybrid were motile they seemed to perform similarly to those of Capercaillie and Black Grouse. Therefore, reduced fertility of the hybrids can rather be linked to disturbances in sperm acrosome or nucleus. However, this hypothesis needs future testing.

In conclusion, the quality of the semen was lower in the hybrid than in Capercaillie and Black Grouse. However, despite reduced quality of semen, a population of viable spermatozoa with good motility characteristics present in the hybrid semen suggests that the hybrid can be fertile, which agrees with previous data indicating limited fertility of these hybrids.

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Teerikukon ja koppelon risteymän siemennesteen laatu

Tutkijat selvittivät teeren ja metson koirasristeymäyksilön siemennesteen laatua vertailemalla neljään teeri- ja yhteen metsokukkoon. Risteymältä löytyi siittiöitä, jotka kykenivät liikkumaan kantalajien siittiöiden tapaan ja olivat morfologisesti samankaltaisia. Siemennesteen laatu oli kuitenkin heikompi, mikä näkyi eritoten alhaisessa siittiöpitoisuudessa ja normaalimuotoisten siittiöiden osuudessa. Tutkijat liittävät risteymien yleisen alentuneen hedelmällisyyden siittiöiden kärkiosan rakenteeseen mutta korostavat aiheen vaativan lisätutkimuksia. Koska risteymät tuottavat normaalimuotoisia ja hyvin liikkuvia siittiöitä, ne myös ilmeisesti kykenevät ainakin jossain määrin lisääntymään.

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