Characteristics of semen collected from gander included in the genetic resources conservation program

Ewa Łukaszewicz,[1] Artur Kowalczyk, and Anna Jerysz

Division of Poultry Breeding, Institute of Animal Breeding, Wroclaw University of Environmental and Life Sciences, Chełmońskiego 38c, 51-630 Wrocław, Poland

ABSTRACT Conservative breeding ex situ in vivo is one of the most popular methods of creating genetic reserves. Unfortunately, keeping animals in small closed populations leads to inbreeding which reduces their reproductive capacity. The aim of the study was to characterize the sperm quality of 6 genetic groups of geese (northern and southern breeds) kept in Poland for many generations as genetic reserve flocks. Each breed was represented by 10 randomly selected 1-yr-old ganders, semen was collected 14 times, individually from each male, and the number of positive reactions (ended with ejaculation), semen volume, sperm concentration, and morphology were assessed. The obtained results showed a significant difference between breeds and individuals of the same group, both in males' reaction and semen quantitative and qualitative traits. From the northern breeds 193 ejaculates were obtained in total (i.e., 45.9% of all attempts), from the southern breeds 242 ejaculates (57.6%). The volume of single ejaculate varied from 0.01 mL (one drop allowing only histological smear and sperm morphology evaluation) to 0.65 mL; sperm concentration varied from $23.0 \times 10^6 mL^{-1}$ to $2376.0 \times 10^6 mL^{-1}$; the amount of total live sperm was at a similar level in all breeds (89.6%−97.7%), while live normal cells ranged between 15.2% and 67.9% depending on breed and individuals. When keeping the genetic reserves ex situ in vivo, attention should be paid to the quality of semen and males that are poor in this respect should be eliminated, in order not to lead to an excessive weakening of the reproductive capacity of the flocks covered by the genetic resources protection program.

Key words: biodiversity, genetic reserve, goose, semen, sperm morphology

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INTRODUCTION

Currently, nearly half of the domesticated bird species and breeds are threatened with extinction (Blesbois et al., 2007), caused, among others, by through restructuring of rural areas, intensive livestock production and diseases. Endangered bird populations are most often protected by the in vivo method (ex situ and in situ), consisting in the protection of live animals in their natural or artificially created, but close to natural environment (Blesbois et al., 2007, 2008), however, it is not a sufficiently effective method of conserving animal genetic resources. In vivo methods carry the risk of losing valuable genes due to increased inbreeding in small, closed populations, outbreaks of pathogens, or some natural disasters.

A significant decrease in the number of local poultry breeds in Poland, including goose, has been observed in the last few decades. The phenotypic patterns of native goose breeds kept in different parts of Poland were determined in the 1960s, (Książkiewicz, 2007), and a program for the protection of genetic resources, whose basic goal is to maintain the genetic balance at a constant level, while maintaining the characteristic phenotypic features of both sexes of particular bird population has been initiated (www.bioroznorodnosc.izoo.krakow.pl). In the early 1970s, the first protection programs for local goose breeds consisting in the creation and maintenance of a gene pool were implemented (Krawczyk et al., 2014; Dobrzański et al., 2019).

The genetic resources protection program covers 14 breeds of goose classified by FAO as global genetic resources subject to protection (B.D. Scherf ed., 2000). Depending on their origin, goose breeds were classified as northern breeds: Kartuska (Ka), Pomorska (Po), Rypińska (Ry), Suwalska (Su), southern: Kielecka (Ki), Lubelska (Lu), Podkarpacka (Pd) Biłgorajska (Bi), Zatorska (ZD-1), and foreign ones: Garbonosa (Ga), Landes (LsD-01), Roman (Ro), Słowacka (Sł),

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[1]Corresponding author: ewa.lukaszewicz@upwr.edu.pl

Kubańska (Ku). These breeds come from both, the Greylag Goose (*Anser anser*) and the Swan Goose (*Anser cygnoides*) (Książkiewicz, 2007; Dobrzański et al., 2019). Similar conservation programs have also been introduced in Hungary, a country with long-lasting tradition in goose breeding. In total, 22 goose genotypes are under protection, of which 15 (68%) are native Hungarian goose (Bodi et al., 2019).

Until recently, studies on goose conservative flocks included mainly the assessment of embryo karyotype and chromosomal abnormalities, DNA and serum protein polymorphism, temporal trends in the reproductive traits, chemical composition, morphological and qualitative characteristics of eggs and meat (Mazanowski et al., 2005; 2006a, b; Książkiewicz, 2007). It is well known that there is an increase in homozygosity level in small closed populations, which reduces the reproductive capacity of inbred individuals (Graczyk et al., 2018). In the case of birds, the most effective method of ex situ in vitro biodiversity protection is the creation of sperm banks (Saint Jalme, 2003; Łukaszewicz et al., 2011), therefore, sperm quality plays a very important role in the successful cryopreservation process. Its monitoring, in parallel with the assessment of other features characteristic for a given genetic group, is extremely important. Taking the above into account, we decided to carry out a macro- and microscopic evaluation of fresh semen quality of selected goose breeds, and in case of positive results, to continue our research on the possibility of freezing and choosing the most advantageous method of semen cryopreservation. It should be mentioned that already in the early 1980s, we conducted the first studies on the evaluation of fresh and subsequent stages of the freeze-thaw process of semen obtained from Kubańska goose (*Anser cygnoides*), covered by the genetic resources protection program (Chełmońska et al., 1984) and later, from White Italian goose (Łukaszewicz, 2002).

A large number of techniques and tests are available to determine the reproductive potency of individuals of both sexes, including sperm, the quality of which is the best indicator of male reproductive performance. For a practical reason, as well as economic aspects and availability of specific test methods, it is important that the evaluation method is reliable, but also simple, cheap, and possible to be made in a farm condition. Our to date research shows that one of the most reliable methods of semen quality assessment is not the determination of total amount of sperm in the ejaculate, but the number of live properly formed sperm, because only such sperm have the highest potency to fertilize an egg (Bakst et al., 1994; Liu et al., 2008; Zawadzka et al., 2015; Łukaszewicz et al., 2020a,b,c).

Considering the necessity of biodiversity protection, not only of free-living species, and the specificity of goose reproduction, the aim of the study was to assess the basic quantitative and qualitative traits of semen collected from ganders protected as genetic reserve breeds.

MATERIAL AND METHODS

Birds and Management

The experiment was conducted on ganders of 6 local goose breeds kept as genetic reserve flocks, including 3 northern breeds: Kartuska (Ka), Rypińska (Ry) and Suwalska (Su) and 3 southern ones: Kielecka (Ki), Lubelska (Lu) and Podkarpacka (Pd). Birds were delivered from the Waterfowl Genetic Resource Station of the National Research Institute of Animal Production in Dworzyska, Poland. Each breed was represented by 10 randomly selected, 1-yr-old males. During the experimental period, males were kept individually in large boxes (80 cm high × 100 × 100 cm) with a deep litter, in an unheated room, with natural microclimatic conditions (temperature and light) and gravity ventilation. At the farm location where the birds were kept (52°2124N, 16°313E), during the experimental period, the day length increased from 10 h in the middle of February up to 14 h in the middle of April, while the temperature inside the building varied between 8°C and 12°C. Every male obtained daily 350 to 400 g of commercial food for breeding goose and had free access to fresh, good quality water.

Semen Collection

One month prior to the onset of semen evaluation (middle of January), ganders were placed in boxes and accustomed to semen collection procedure (catching, massage, presence of operators). Semen was collected from each male by dorsoabdominal massage twice a week, from mid-February to mid-April (from 2 breeds in 1 d). Clean samples (free from uric or fecal contamination) were subjected to further microscopic analysis. The following procedure was maintained during semen collection: birds were operated by the same persons, caught and massaged in the morning (before feeding), the time elapsed from the collection of the first ejaculate to semen preparation for testing did not exceed 20 to 25 min.

Semen Evaluation

The ejaculate volume (using an automatic pipette, with accuracy to 0.01 mL), sperm concentration (spectrophotometric method using the Accucel photometer, IMV Technologies, L'Aigle, France) and morphology (on the basis of live stained nigrosine-eosin histological smears) were assessed individually. In every smear, 300 cells were examined at 1,250 × under a light microscope (Jenaval, Carl Zeiss, Jena, Germany). Spermatozoa were attributed to 7 categories: 6 of them, named "total live," were as follows: 1) morphologically normal (typical spindle-shaped head and well-marked acrosome); 2) bulb-head; 3) crooked-neck; 4) midpiece deformed (swelling, ragged, or lack of midpiece); 5) spermatids (immature forms); 6) spermatozoa with other deformities (not included in any of the previous category); spermatozoa pink stained by eosin were indicated as dead

7). The results of morphological evaluation were expressed as the percentage of particular categories of spermatozoa (300 cells = 100%). For better males' comparison in terms of semen quality, the semen quality factor (**SQF**) involving ejaculate volume, sperm concentration, and percentage of live normal sperm was calculated (Łukaszewicz, 2006).

Statistical Analysis

Obtained data were analyzed by one-way ANOVA. The significance of differences between the examined males and breeds was determined using Tukey post-hoc test for unequal counts (Statistica, version 12.5 StatSoft, Inc., Kraków, Poland). For SQF statistical analyzes, only males from which at least 5 ejaculates were collected and only those who had a complete analysis, that is, sperm volume, concentration, and morphology, were considered.

RESULTS

The average data of reactions to semen collection procedure and semen quality of ganders from northern and southern breeds as well as the differences between the breeds are presented in Table 1, Tables 2–4 present the data of the northern goose breeds, while Tables 5–7 the southern breeds. During the experimental period, 840 semen collection attempts were made in total, 420 in each regional goose group (14 attempts for each male). The obtained results showed a significant difference between the breeds and individuals of the same group, both in males' reaction and semen quantitative and qualitative traits.

Male Reactions to the Semen Collection Procedure

From the northern goose breeds, 193 ejaculates were obtained, that is, 45.9% of all attempts. The best reactions were stated in the Suwalska (Su) ganders (80; 57.1%), the poorest in Rypińska (Ry) (51; 36.4%) (Table 1), although the ♂8 of the Ry breed had the highest libido, producing semen 13 times (92.9%) out of 14 attempts of collection. In every breed of this regional group, there were 1 to 3 males from which no ejaculate was collected (Tables 2–4). In the southern goose breeds, male reactions were more effective−−242 ejaculates were collected (57.6% of all attempts). The largest number of ejaculates (82; 58.6%) was obtained from Lubelska (Lu) males and slightly less (80; 57.1%) from Kielecka (Ki) and Podkarpacka (Pd) ganders (Table 1). In this regional group, all males responded to the massage procedure, and the number of positive reactions (ended with ejaculation) ranged from 1 (7.1%) to 14 (100%) (Tables 5–7).

Table 1. Semen characteristics of six goose breeds involved in Polish genetic resources conservation program (means ± SD).

Goose breed*)	Obtained samples [no] (%)	Ejaculate volume [mL]	Sperm concentration [n x10^6mL^{-1}]	Sperm morphology [%]								No of SQF evaluations
				Live in total	Live normal	Bulb head	Sperma-tids	Bent neck	Other deform.	Dead sperm	Deform. together	SQF	
North goose breeds													
Kartuska (Ka)	62 (44.3%)	$0.14^{c} \pm 0.05$	426.9 ± 357.2	94.0 ± 3.9	$53.8^{a} \pm 11.3$	$20.3^{bc} \pm 8.4$	$4.8^{a} \pm 3.4$	$8.0^{b} \pm 4.0$	$7.2^{bc} \pm 3.8$	6.0 ± 3.9	$35.5^{b} \pm 9.4$	$31.2^{b} \pm 29.6$	36
Rypińska (Ry)	51 (36.4%)	$0.41^{a} \pm 0.29$	482.7 ± 403.4	94.0 ± 3.0	$49.7^{a} \pm 7.6$	$21.5^{bc} \pm 6.6$	$3.4^{b} \pm 2.9$	$8.8^{b} \pm 3.2$	$10.5^{ad} \pm 3.5$	6.0 ± 3.0	$40.7^{ab} \pm 6.3$	$99.7^{a} \pm 98.3$	21
Suwalska (Su)	80 (57.1%)	$0.20^{b} \pm 0.08$	476.4 ± 328.7	94.8 ± 3.5	$52.8^{a} \pm 14.6$	$22.4^{bc} \pm 8.7$	$4.5^{a} \pm 3.3$	$6.1^{c} \pm 3.0$	$9.1^{cd} \pm 4.4$	5.2 ± 3.5	$37.5^{b} \pm 11.5$	$54.9^{ab} \pm 45.0$	39
South goose breeds													
Kielecka (Ki)	80 (57.1%)	$0.20^{b} \pm 0.09$	687.8 ± 410.7	93.5 ± 3.9	$43.6^{b} \pm 13.5$	$26.6^{a} \pm 7.9$	$6.3^{a} \pm 5.2$	$6.7^{bc} \pm 2.6$	$10.3^{ac} \pm 4.4$	6.5 ± 3.9	$43.6^{a} \pm 9.6$	$68.3^{ab} \pm 63.9$	44
Lubelska (Lu)	82 (58.6%)	$0.16^{b} \pm 0.05$	709.3 ± 913.2	93.3 ± 3.8	$43.7^{b} \pm 17.2$	$22.5^{b} \pm 10.7$	$3.7^{b} \pm 2.7$	$11.6^{a} \pm 5.9$	$11.7^{a} \pm 7.7$	6.7 ± 3.8	$44.3^{a} \pm 16.4$	$60.9^{ab} \pm 84.3$	35
Podkarpacka (Pd)	80 (57.1%)	$0.19^{b} \pm 0.06$	751.9 ± 603.2	93.9 ± 3.4	$52.7^{a} \pm 12.5$	$18.4^{c} \pm 8.3$	$6.3^{a} \pm 4.8$	$6.3^{c} \pm 2.9$	$10.3^{ac} \pm 3.1$	6.1 ± 3.4	$35.0^{b} \pm 9.7$	$89.0^{a} \pm 107.8$	34

*Each goose breed was represented by 10 ganders; for each male 14 semen collection attempts were performed, 140 semen samples = 100%.
a–dMean values in columns followed by different superscripts differ significantly ($P < 0.05$).
SQF, Semen Quality Factor = sperm concentration ($n \times 10^6$ mL^{-1}) × ejaculate volume (mL) × live normal spermatozoa (%)/100%.

Table 2. Characteristics of semen collected individually from ganders of Kartuska (Ka) goose breed (means ± SD).

Male's number	Obtained samples [no] (%)	Ejaculate volume [mL]	Sperm concentration [n ×10^{6}mL^{-1}]	Sperm morphology [%]								SQF	No of SQF evaluations
				Live in total	Live normal	Bulb head	Sperma-tids	Bent neck	Other deform.	Dead sperm	Deform. together		
Ka 1	9 (64.3)	0.19^{a} ± 0.05	233.2 ± 234.7	94.6^{ab} ± 7.4	53.0^{cd} ± 7.1	18.9^{bc} ± 4.1	9.1^{a} ± 3.2	6.8^{b} ± 2.0	6.8^{abc} ± 2.3	5.4^{ab} ± 7.4	32.6^{b} ± 5.8	22.5^{ab} ± 18.3	5
Ka 2	10 (71.4)	0.10^{b} ± 0.02	647.0 ± 470.4	94.8^{ab} ± 3.0	67.2^{a} ± 8.4	14.5^{c} ± 5.2	0.8^{c} ± 0.5	5.1^{c} ± 1.9	7.4^{ab} ± 3.3	5.2^{ab} ± 3.0	26.9^{b} ± 6.5	41.1^{ab} ± 28.6	6
Ka 3	10 (71.4)	0.18^{a} ± 0.03	607.0 ± 313.7	93.8^{ab} ± 3.6	58.3^{bc} ± 6.3	14.1^{c} ± 4.1	3.7^{b} ± 1.6	13.2^{a} ± 3.4	4.5^{c} ± 1.1	6.2^{ab} ± 3.6	31.8^{b} ± 4.4	64.5^{a} ± 42.0	6
Ka 4	3^{x,y} (21.4)	0.09 ± 0.01	453.7 ± 522.0	94.1 ± 3.0	48.3 ± 11.9	29.4 ± 12.8	3.1 ± 1.8	5.8 ± 2.8	7.4 ± 4.2	5.9 ± 3.0	42.7 ± 12.2	19.2$^{\#}$ ± 22.3	3
Ka 5	7^{y} (50.0)	0.06^{b} ± 0.01	358.3 ± 255.4	94.2^{ab} ± 2.5	61.9^{ab} ± 7.4	17.1^{c} ± 5.4	4.4^{b} ± 2.1	4.8^{c} ± 1.8	6.1^{bc} ± 1.6	5.8^{ab} ± 2.5	28.0^{b} ± 7.1	11.3$^{\#}$ ± 5.6	3
Ka 6	9 (64.3)	0.18^{a} ± 0.06	424.0 ± 257.5	95.5^{a} ± 2.0	47.5^{d} ± 7.4	24.0^{b} ± 7.8	3.7^{b} ± 2.0	11.6^{a} ± 3.1	8.7^{a} ± 2.7	4.5^{a} ± 2.0	44.3^{a} ± 5.7	32.3^{ab} ± 16.0	6
Ka 7	7 (50.0)	0.13^{ab} ± 0.02	187.0 ± 119.0	90.9^{b} ± 2.2	42.1^{d} ± 7.8	30.7^{a} ± 7.8	8.6^{a} ± 3.3	4.5^{c} ± 1.2	5.0^{bc} ± 1.4	9.1^{b} ± 2.2	40.1^{a} ± 7.5	10.1^{b} ± 4.9	5
Ka 8	4^{x,y} (28.6)	0.14 ± 0.01	622.0 ± 814.5	91.8 ± 3.6	42.9 ± 8.9	18.3 ± 4.3	4.8 ± 0.8	8.9 ± 1.7	16.9 ± 5.3	8.3 ± 3.6	44.1 ± 56.8	42.0$^{\#}$ ± 56.8	2
Ka 10	3^{x,y} (21.4)	0.14 ± 0.06	186.5 ± 86.9	96.2 ± 3.2	43.5 ± 6.1	30.7 ± 4.9	5.9 ± 2.4	9.1 ± 2.8	7.1 ± 3.6	3.8 ± 3.2	46.8 ± 2.4	10.5$^{\#}$ ± 0.6	2

$^{a-c}$Mean values in columns followed by different superscripts differ significantly ($P < 0.05$).
^{x}Four or less histological smears for sperm morphology evaluations were obtained therefore the results were not considered in the statistical analysis.
^{y}Four or less sperm volume and concentration assessments were obtained therefore the results were not considered in the statistical analysis.
$^{\#}$Less than five SQF analyses could be calculated therefore the results were not considered in the statistical analysis.
SQF, Semen Quality Factor = sperm concentration ($n \times 10^6$ mL^{-1}) × ejaculate volume (mL) × live normal spermatozoa (%)/100%.
From gander no 9 any semen sample could be collected, therefore it is not included in the above table.

Table 3. Characteristics of semen collected individually from ganders of Rypińska (Ry) goose breed (means ± SD).

Male's number	Obtained samples [no] (%)	Ejaculate volume [mL]	Sperm concentration [n ×10^{6}mL^{-1}]	Sperm morphology [%]								SQF	No of SQF evaluations
				Live in total	Live normal	Bulb head	Spermatids	Bent neck	Other deform.	Dead sperm	Deform. together		
Ry 1	3^{x,y} (21.4)	0.01 ± 0.0	0.00 ± 0.0	94.9 ± 3.8	37.6 ± 7.4	24.2 ± 4.4	12.8 ± 1.8	8.3 ± 3.0	12.0 ± 3.5	5.1 ± 3.8	44.6 ± 2.4	0.00$^{\#}$ ± 0.0	0
Ry 5	5^{y} (35.7)	0.01 ± 0.0	0.00 ± 0.0	95.7 ± 0.7	56.9^{a} ± 3.5	16.1^{bc} ± 4.2	3.2^{ab} ± 0.4	8.7^{b} ± 2.2	10.8^{b} ± 2.7	4.3^{a} ± 0.7	35.6^{b} ± 3.4	0.00$^{\#}$ ± 0.0	0
Ry 6	4^{x,y} (28.6)	0.01 ± 0.0	0.00 ± 0.0	93.8 ± 3.0	56.3 ± 10.5	18.3 ± 7.5	2.4 ± 1.7	8.3 ± 0.3	8.6 ± 1.9	6.3 ± 3.0	35.1 ± 8.4	0.00$^{\#}$ ± 0.0	0
Ry 7	10 (71.4)	0.20 ± 0.05	436.2 ± 431.9	95.2 ± 2.2	51.8^{ab} ± 5.6	21.9^{ab} ± 6.1	1.8^{b} ± 0.9	10.6^{ab} ± 2.8	9.2^{b} ± 2.9	4.8^{a} ± 2.2	41.6^{ab} ± 6.2	54.8 ± 66.6	5
Ry 8	13 (92.9)	0.31 ± 0.02	844.6 ± 483.0	92.6 ± 2.6	47.9^{b} ± 5.9	25.6^{a} ± 4.9	3.9^{a} ± 2.3	5.4^{c} ± 1.7	9.9^{b} ± 3.2	7.4^{a} ± 2.6	40.9^{ab} ± 5.1	126.3 ± 105.0	5
Ry 9	10 (71.4)	0.50 ± 0.30	242.9 ± 126.7	93.1 ± 3.7	49.3^{b} ± 7.5	22.5^{a} ± 6.1	2.0^{b} ± 0.9	9.9^{ab} ± 2.8	9.3^{b} ± 2.8	6.9^{b} ± 3.7	41.7^{ab} ± 6.7	70.1 ± 58.4	5
Ry 10	6 (42.9)	0.61 ± 0.38	420.2 ± 326.7	95.2 ± 3.5	48.6^{b} ± 7.3	15.2^{c} ± 7.5	3.1^{ab} ± 1.2	11.9^{a} ± 3.6	16.4^{a} ± 2.5	4.8^{ab} ± 3.5	43.4^{a} ± 8.0	139.6 ± 134.2	6

$^{a-c}$Mean values in columns followed by different superscripts differ significantly ($P < 0.05$).
^{x}Four or less histological smears for sperm morphology evaluations were obtained therefore the results were not considered in the statistical analysis.
^{y}Four or less sperm volume and concentration assessments were obtained therefore the results were not considered in the statistical analysis.
$^{\#}$Less than five SQF analyses could be calculated therefore the results were not considered in the statistical analysis.
SQF, Semen Quality Factor = sperm concentration ($n \times 10^6$ mL^{-1}) ejaculate volume (mL) × live normal spermatozoa (%)/100%.
From ganders no 2, 3, and 4 any semen sample could be collected, therefore they are not included in the above table.

Table 4. Characteristics of semen collected individually from ganders of Suwalska (Su) goose breed (means ± SD).

Male's number	Obtained samples [no] (%)	Ejaculate volume [mL]	Sperm concentration [n ×10^{6}mL^{-1}]	Sperm morphology [%]									No of SQF evaluations
				Live in total	Live normal	Bulb head	Spermatids	Bent neck	Other deform.	Dead sperm	Deform. together	SQF	
Su 1	6[y] (42.9)	0.01 ± 0.0	0.00 ± 0.0	93.2[b] ± 3.0	40.1[c] ± 14.7	26.8[ab] ± 11.6	5.4[c] ± 1.7	7.2[ab] ± 2.7	13.7[b] ± 4.3	6.8[b] ± 3.0	47.7[a] ± 12.8	0.00[#] ± 0.0	0
Su 3	10[y] (71.4)	0.01 ± 0.0	0.00 ± 0.0	95.4[a] ± 2.7	41.5[c] ± 10.8	29.8[a] ± 10.0	11.0[a] ± 2.5	4.8[bc] ± 1.6	8.3[cd] ± 3.4	4.6[a] ± 2.7	42.9[a] ± 9.8	0.00[#] ± 0.0	0
Su 4	12 (85.7)	0.23[ab] ± 0.09	278.8[b] ± 65.7	96.9[a] ± 1.4	67.9[a] ± 5.8	14.0d ± 3.2	0.9[f] ± 0.5	5.7[bc] ± 2.2	8.4[cd] ± 2.0	3.1[a] ± 1.4	28.1[b] ± 5.3	48.1[ab] ± 26.7	6
Su 5	8 (57.1)	0.24[ab] ± 0.01	564.3[ab] ± 459.7	95.5[a] ± 2.1	44.5[c] ± 9.6	27.6[ab] ± 7.6	4.7[cd] ± 1.4	9.7[a] ± 1.7	9.0[c] ± 2.1	4.5[a] ± 2.1	46.3[a] ± 7.6	62.4[ab] ± 53.2	6
Su 6	6 (42.9)	0.14[b] ± 0.04	243.8[b] ± 135.5	94.9[a] ± 2.0	44.2[c] ± 8.8	23.1[abc] ± 8.7	5.2[c] ± 1.0	9.1[a] ± 2.8	13.4[b] ± 2.5	5.1[a] ± 2.0	45.6[a] ± 8.8	13.9[b] ± 6.7	6
Su 7	12 (85.7)	0.26[a] ± 0.10	590.9[ab] ± 265.0	96.1[ab] ± 2.8	59.6[a] ± 9.3	23.7[abc] ± 6.0	2.8[e] ± 1.2	4.1[c] ± 2.2	5.9[d] ± 2.3	3.9[ab] ± 2.8	33.7[b] ± 7.8	90.0[a] ± 45.3	8
Su 8	5[y] (35.7)	0.30 ± 0.00	243.0 ± 104.7	88.9[c] ± 5.9	29.9[d] ± 8.6	22.7[abc] ± 7.2	7.5[b] ± 0.8	9.4[a] ± 4.9	19.3[a] ± 2.6	11.1[c] ± 5.9	51.4[a] ± 8.2	29.6[#] ± 0.0	1
Su 9	9 (64.3)	0.17[ab] ± 0.06	318.2[b] ± 127.8	93.0[b] ± 3.6	55.1[b] ± 10.4	21.0[bcd] ± 7.4	3.7[de] ± 1.1	5.7[bc] ± 2.1	7.6[cd] ± 2.5	7.0[b] ± 3.6	34.3[b] ± 9.9	30.5[ab] ± 14.4	6
Su 10	12 (85.7)	0.13[b] ± 0.04	863.2[a] ± 331.4	94.8[ab] ± 3.8	64.1[ab] ± 6.4	18.0[cd] ± 6.5	3.1[e] ± 1.1	3.8[c] ± 1.6	5.9[d] ± 2.8	5.2[ab] ± 3.8	27.7[b] ± 6.8	77.2[ab] ± 56.6	6

[a-f]Mean values in columns followed by different superscripts differ significantly ($P < 0.05$).
[y]Four or less sperm volume and concentration assessments were obtained therefore the results were not considered in the statistical analysis.
[#]Less than five SQF analyses could be calculated therefore the results were not considered in the statistical analysis.
SQF, Semen Quality Factor = sperm concentration ($n \times 10^6$ mL^{-1}) ejaculate volume (mL) × live normal spermatozoa (%)/100%.
From gander no 2 any semen sample could be collected, therefore it is not included in the above table.

Table 5. Characteristics of semen collected individually from ganders of Kielecka (Ki) goose breed (means ± SD)

Male's number	Obtained samples [no] (%)	Ejaculate volume [mL]	Sperm concentration [n ×10^{6}mL^{-1}]	Sperm morphology [%]									No of SQF evaluations
				Live in total	Live normal	Bulb head	Spermatids	Bent neck	Other deform.	Dead sperm	Deform. together	SQF	
Ki 1	9 (64.3)	0.13[b] ± 0.1	856.0[ab] ± 204.6	89.6[c] ± 6.8	38.2[bc] ± 10.8	30.4[a] ± 11.4	4.8[bc] ± 2.1	5.7[cd] ± 2.3	10.6[b] ± 3.9	8.4[c] ± 4.7	46.7[ab] ± 9.4	46.1[bc] ± 21.9	5
Ki 2	9[y] (64.3)	0.35 ± 0.0	107.0 ± 0.0	94.0[ab] ± 3.4	47.1[ab] ± 9.6	29.1[ab] ± 2.6	3.6[c] ± 4.9	4.7[d] ± 1.9	9.5[b] ± 4.4	6.0[ab] ± 3.4	43.3[ab] ± 5.0	31.3[#] ± 0.0	1
Ki 3	2[y] (14.3)	0.08 ± 0.00	449.0 ± 323.3	91.7 ± 1.9	49.0 ± 0.0	23.0 ± 8.5	4.3 ± 1.4	6.3 ± 1.4	9.0 ± 3.8	8.3 ± 1.9	38.3 ± 3.3	19.3[#] ± 15.4	2
Ki 4	5[y] (35.7)	0.09 ± 0.01	417.6 ± 241.2	91.8[bc] ± 4.3	26.6[d] ± 13.8	22.7[b] ± 7.8	13.9[a] ± 2.1	11.1[a] ± 2.0	18.3[a] ± 4.8	7.4[bc] ± 4.3	52.1[a] ± 12.1	12.1[#] ± 0.7	3
Ki 5	7 (50.0)	0.19a[b] ± 0.04	257.0[c] ± 306.4	96.7[a] ± 1.5	44.0[ab] ± 10.6	31.4[a] ± 7.5	6.0[bc] ± 4.8	7.2[bc] ± 2.6	8.2[b] ± 2.6	3.3[a] ± 1.5	46.8[ab] ± 9.2	25.1[c] ± 28.0	5
Ki 6	1[x;y] (7.1)	0.01 ± 0.0	0.00 ± 0.0	97.3 ± 0.0	56.3 ± 0.0	18.0 ± 0.0	4.3 ± 0.0	6.0 ± 0.0	12.7 ± 0.0	2.7 ± 0.0	36.7 ± 0.0	0.00[#] ± 0.0	0
Ki 7	8 (57.1)	0.29[a] ± 0.1	425.0[bc] ± 170.6	93.5[ab] ± 3.1	30.9[cd] ± 8.7	26.5[ab] ± 7.9	16.1[a] ± 5.9	5.1[cd] ± 1.3	14.9[a] ± 4.8	6.5[ab] ± 3.1	46.5[ab] ± 8.1	40.0[bc] ± 23.9	7
Ki 8	12 (85.7)	0.24[ab] ± 0.10	1263.5[a] ± 315.2	94.3[ab] ± 3.2	52.9[a] ± 3.5	24.3[ab] ± 7.8	3.3[c] ± 0.9	5.9[bcd] ± 2.1	7.9[b] ± 2.8	5.7[ab] ± 3.2	38.1[b] ± 11.9	184.6[a] ± 74.4	6
Ki 9	13 (92.9)	0.21[ab] ± 0.1	910.8[ab] ± 233.9	96.1[ab] ± 2.5	54.9[a] ± 9.3	23.6[ab] ± 7.5	2.7c ± 0.9	7.1[bc] ± 2.9	7.9[b] ± 2.2	3.9[ab] ± 2.5	38.5[b] ± 8.7	103.8[b] ± 30.6	8
Ki 10	14 (100.0)	0.20[ab] ± 0.1	644.7[bc] ± 387.3	89.8[c] ± 3.3	36.4[bcd] ± 9.0	27.8[ab] ± 6.8	7.1[b] ± 2.2	8.0[b] ± 1.3	10.5[b] ± 3.4	10.3[c] ± 3.3	46.3[ab] ± 8.4	46.4[bc] ± 29.6	7

[a-d]Mean values in columns followed by different superscripts differ significantly ($P < 0.05$).
[x]Four or less histological smears for sperm morphology evaluations were obtained therefore the results were not considered in the statistical analysis.
[y]Four or less sperm volume and concentration assessments were obtained therefore the results were not considered in the statistical analysis.
[#]Less than five SQF analyses could be calculated therefore the results were not considered in the statistical analysis.
SQF, Semen Quality Factor = sperm concentration ($n \times 10^6$ mL^{-1}) ejaculate volume (mL) × live normal spermatozoa (%)/100%.

Table 6. Characteristics of semen collected individually from ganders of Lubelska (Lu) goose breed (means ± SD).

Male's number	Obtained samples [no] (%)	Ejaculate volume [mL]	Sperm concentration [n ×10^{6}mL^{-1}]	Sperm morphology [%]								SQF	No of SQF evaluations
				Live in total	Live normal	Bulb head	Spermatids	Bent neck	Other deform.	Dead sperm	Deform. together		
Lu 1	11 (78.6)	0.20^{a} ± 0.03	800.5 ± 853.3	92.5^{ab} ± 5.7	37.1^{c} ± 14.6	26.5^{ab} ± 14.3	3.2^{c} ± 1.0	15.5^{a} ± 4.1	10.4 ± 2.4	7.5^{ab} ± 5.7	45.3^{bc} ± 15.5	58.9 ± 65.4	6
Lu 2	11 (78.6)	0.12^{b} ± 0.05	313.8 ± 189.1	94.2^{ab} ± 4.1	54.4^{ab} ± 13.5	16.7^{c} ± 8.9	1.0^{d} ± 0.8	9.8^{b} ± 3.4	12.4 ± 2.6	5.8^{ab} ± 4.1	38.8^{bc} ± 12.4	30.6 ± 24.6	5
Lu 3	13 (92.9)	0.11^{b} ± 0.04	581.6 ± 117.8	92.8^{ab} ± 3.3	51.4^{ab} ± 11.9	22.4^{ab} ± 7.8	3.3^{bc} ± 1.6	7.7^{b} ± 3.1	8.1 ± 2.7	7.2^{ab} ± 3.3	38.1^{bc} ± 10.7	38.7 ± 20.9	5
Lu 4	11 (78.6)	0.19 ± 0.04	1192.5 ± 673.5	94.2^{ab} ± 2.8	61.0^{a} ± 8.4	13.2^{c} ± 6.0	3.4^{bc} ± 2.2	7.4^{b} ± 2.6	9.1 ± 3.5	5.8^{ab} ± 2.8	29.7^{c} ± 7.8	215.0$^{\#}$ ± 69.3	4
Lu 5	9 (64.3)	0.15 ± 0.06	278.5 ± 99.9	90.5^{b} ± 3.7	45.0^{bc} ± 6.3	18.6^{bc} ± 7.4	1.7^{d} ± 0.9	16.3^{a} ± 7.1	8.9 ± 2.7	9.5^{b} ± 3.7	43.7^{bc} ± 8.7	20.8$^{\#}$ ± 155.9	4
Lu 6	3$^{x;y}$ (21.4)	0.20 ± 0.0	269.0 ± 0.0	93.1 ± 2.0	49.1 ± 14.0	18.4 ± 9.1	6.2 ± 1.6	10.8 ± 2.5	8.6 ± 2.5	6.9 ± 2.0	37.8 ± 11.1	34.2$^{\#}$ ± 0.0	1
Lu 7	6^{y} (42.9)	0.01 ± 0.0	0.00 ± 0.0	91.1^{ab} ± 3.8	15.2^{a} ± 8.0	26.4^{ab} ± 9.9	6.3^{a} ± 2.4	9.7^{b} ± 5.5	33.4 ± 13.2	8.9^{ab} ± 3.8	61.0^{ab} ± 27.4	0.00$^{\#}$ ± 0.0	0
Lu 8	3$^{x;y}$ (21.4)	0.18 ± 0.04	121.0 ± 39.6	97.1 ± 1.4	52.9 ± 14.8	18.4 ± 7.5	3.9 ± 2.5	12.4 ± 6.0	9.4 ± 2.5	2.9 ± 1.4	40.3 ± 15.4	12.5$^{\#}$ ± 0.6	2
Lu 9	11 (78.6)	0.15 ± 0.01	214.8 ± 60.4	94.5^{a} ± 2.1	25.5^{d} ± 10.2	33.4^{a} ± 6.6	4.8^{b} ± 1.3	18.3^{a} ± 4.4	12.5 ± 4.3	5.5^{a} ± 2.1	64.2^{a} ± 10.5	10.9$^{\#}$ ± 3.9	4
Lu 10	4$^{x;y}$ (28.6)	0.14 ± 0.06	393.0 ± 527.8	95.2 ± 1.9	38.7 ± 8.5	32.9 ± 7.0	11.1 ± 1.6	3.8 ± 1.2	8.8 ± 1.9	4.8 ± 1.9	45.4 ± 7.9	22.7$^{\#}$ ± 31.2	4

$^{a\text{-}d}$Mean values in columns followed by different superscripts differ significantly ($P < 0.05$).
^{x}Four or less histological smears for sperm morphology evaluations were obtained therefore the results were not considered in the statistical analysis.
^{y}Four or less sperm volume and concentration assessments were obtained therefore the results were not considered in the statistical analysis.
$^{\#}$Less than five SQF analyses could be calculated therefore the results were not considered in the statistical analysis.
SQF, Semen Quality Factor = sperm concentration ($n \times 10^6$ mL^{-1}) ejaculate volume (mL) × live normal spermatozoa (%)/100%.

Table 7. Characteristics of semen collected individually from ganders of Podkarpacka (Pd) goose breed (means ± SD).

Male's number	Obtained samples [no] (%)	Ejaculate volume [mL]	Sperm concentration [n ×10^{6}mL^{-1}]	Sperm morphology [%]								SQF	No of SQF evaluations
				Live in total	Live normal	Bulb head	Spermatids	Bent neck	Other deform.	Dead sperm	Deform. together		
Pd 1	9 (64.3)	0.18 ± 0.05	48.2 ± 30.1	95.6^{a} ± 1.6	52.5^{bc} ± 6.6	12.9 ± 5.3	13.7^{a} ± 2.5	5.0^{bc} ± 1.5	11.5^{a} ± 2.9	4.4^{a} ± 1.6	29.4^{cd} ± 6.4	4.6$^{\#}$ ± 1.7	4
Pd 2	8 (57.1)	0.14 ± 0.05	442.2^{b} ± 316.2	95.0^{a} ± 1.4	57.1^{b} ± 11.5	16.3 ± 9.2	5.0^{a} ± 2.3	6.7^{ab} ± 1.3	9.9^{ab} ± 1.6	5.0^{a} ± 1.4	33.0^{abcd} ± 8.5	35.5^{b} ± 31.1	5
Pd 3	11 (78.6)	0.19 ± 0.06	502.1^{a} ± 345.2	94.2^{abc} ± 4.2	58.3^{b} ± 9.7	15.7 ± 8.2	4.1^{bc} ± 1.3	7.7^{a} ± 2.6	8.5^{b} ± 1.9	5.8^{abc} ± 4.2	31.8^{bcd} ± 8.4	60.8^{b} ± 51.5	7
Pd 4	13 (92.9)	0.22 ± 0.07	1576.7^{a} ± 562.7	94.9^{ab} ± 2.5	67.4^{a} ± 6.1	13.4 ± 4.8	2.3^{c} ± 1.6	3.4^{c} ± 0.9	8.4^{b} ± 1.5	5.1^{ab} ± 2.5	25.3^{d} ± 5.2	253.6^{a} ± 159.7	6
Pd 5	10^{y} (71.4)	0.19 ± 0.03	1073.0 ± 251.0	92.1^{c} ± 9.0	42.5^{d} ± 9.0	19.8 ± 9.0	13.2^{a} ± 9.0	4.5^{c} ± 9.0	12.1^{a} ± 9.0	7.9^{c} ± 9.0	36.4^{abc} ± 8.3	88.1$^{\#}$ ± 36.1	3
Pd 6	11 (78.6)	0.15 ± 0.02	670.3 ± 528.4	92.5^{bc} ± 1.9	47.2cd ± 9.9	22.8 ± 7.5	3.1^{bc} ± 1.5	7.8^{a} ± 2.7	11.5^{a} ± 2.9	7.5^{bc} ± 1.9	42.1^{ab} ± 8.9	47.9$^{\#}$ ± 33.2	4
Pd 7	1$^{x;y}$ (7.1)	0.01 ± 0.0	0.00 ± 0.0	98.0	17.0	24.0	5.3	18.3	15.3	2.0	57.7	0.00$^{\#}$ ± 0.0	0
Pd 8	1$^{x;y}$ (7.1)	0.01 ± 0.0	0.0 ± 0.0	97.7	57.7	14.3	9.3	6.0	10.3	2.3	30.7	0.00$^{\#}$ ± 0.0	0
Pd 9	4$^{x;y}$ (28.6)	0.01 ± 0.0	0.00 ± 0.0	95.9 ± 2.0	46.1 ± 4.9	21.2 ± 9.3	8.3 ± 2.4	5.1 ± 1.1	15.3 ± 5.0	4.1 ± 2.0	41.6 ± 4.7	0.00$^{\#}$ ± 0.0	0
Pd 10	12 (85.7)	0.21 ± 0.03	857.4^{ab} ± 602.5	93.4^{abc} ± 3.7	47.0^{cd} ± 7.5	25.9 ± 7.7	4.1^{bc} ± 1.2	8.1^{a} ± 2.6	8.4^{b} ± 2.0	6.6^{abc} ± 3.7	42.3^{a} ± 7.1	85.6^{b} ± 56.5	5

$^{a\text{-}d}$Mean values in columns followed by different superscripts differ significantly ($P < 0.05$).
^{x}Four or less histological smears for sperm morphology evaluations were obtained therefore the results were not considered in the statistical analysis.
^{y}Four or less sperm volume and concentration assessments were obtained therefore the results were not considered in the statistical analysis.
$^{\#}$Less than five SQF analyses could be calculated therefore the results were not considered in the statistical analysis.
SQF, Semen Quality Factor = sperm concentration ($n \times 10^6$ mL^{-1}) ejaculate volume (mL) × live normal spermatozoa (%)/100%.

Semen Quantity and Quality

Although semen was collected from each male 14 times, in every group, a complete semen assessment (ejaculate volume, sperm concentration, and morphology, SQF) on the basis of a minimum 5 samples could be performed in only for 6 (Su and Ki) to 3 (Lu) ganders. The other males did not respond to manual stimulation at all, and from none of them a drop of semen (sufficient for histological smear) could be collected from the base of the copulatory organ.

Ejaculate Volumes

The ejaculate volume differed significantly ($P < 0.05$) both, between the regional groups (Table 1) and males within a particular breed (Tables 2, 4–6). In both regional groups, the volume of a single ejaculate (although in several cases perhaps it would be better to say––secretion from the everted copulatory organ) of the evaluated ganders varied from 0.01 mL (one drop allowing only for histological smear and sperm morphology evaluation) to 0.65 mL. Nevertheless, in all groups the average values were on a similar level (Table 1), significant differences between the males were observed in Ka (Table 2), Su (Table 4), Ki (Table 5), and Lu (6). It is interesting that ejaculates obtained from Rypińska ganders, breed with the lowest percentage of positive reaction, had the highest volumes.

Sperm Concentration

The average sperm concentration of evaluated goose breeds is presented in Table 1, in general they were lower in the northern regional group, compared to the southern group, but existing differences were not significant. The concentration of single ejaculates varied from $46.0 \times 10^6 mL^{-1}$ to $1198.0 \times 10^6 mL^{-1}$ (in Ka) or from $23.0 \times 10^6 mL^{-1}$ to $2376.0 \times 10^6 mL^{-1}$ (in Pd), however, significant differences ($P > 0.05$) between the males were found in Su (Table 4), Ki (Table 5), and Pd (Table 7).

Sperm Morphology

Despite goose breed, the average amount of live sperm in total in the freshly collected gander semen was similar ($P > 0.05$), ranging between 93.3% (Lu) and 94.8% (Su), but significant ($P < 0.05$) differences were observed in almost all distinguished morphological forms (Table 1). In a single ejaculate of northern ganders, the live sperm in total constituted from 75.0 to 99.0% (Ka), 86.0–98.7% (Ry) and 78.3–91.0% (Su), and in the group of southern ganders from 80.7 to 91.0% (Ki), 79.3–98.7% (Lu) and 80.0%–99% (Pd). Significant ($P < 0.05$) individual differences were observed in Ka (Table 2), Su (Table 4) and all southern breeds (Tables 5–7). The average content of sperm with intact structure (live normal), that is, the most desirable from the fertilizing potency viewpoint, was much lower and more diverse. Significant differences ($P < 0.05$) were observed between the groups (Table 1) and individual males of the same breed (Tables 2–7). Their amount in the individual ejaculates in northern ganders ranged from 29.7 to 83.7% (in Ka), 32.3–68.3% (Ry) and from 16.0% to 73.0% (in Su), while in southern Ki, Lu, and Pd, it ranged between: 8.7–77.0%; 5.3–73.3% and 17.0–79.7%, respectively.

Among the deformed forms, the bulb head sperm were the most frequent, their percentage in the individual ejaculates of northern ganders varied from 14.0 to 60.0 in Ka semen, 27.3.0–53.3 in Ry and 19.7%–68.3% in Su ganders. In the ejaculates of southern goose breeds, these values were from 19.3 to 67.3% bulb head sperm in Ki ganders' semen, 5.7%–80.7% in Lu and 17.0%–57.7% in Pd breed. Differences between breeds and individuals within the breed were significant ($P < 0.05$). Summing up, all deformed sperm forms shows how large their number is. In the semen of many males, their amount was equal, or even exceeded the number of sperm with normal structure, especially in Ki (Table 5) and Lu (Table 6) males.

Although males of all breeds were sexually mature, there was also a high participation of immature forms––spermatids. The highest (6.3%), average number of this form was found in the semen of Ki and Pd ganders (Tables 5 and 7), varying in individual male ejaculates from 1.0 to 27.3% and 0.3–20.3%, respectively.

Semen Quality Factor

The value of semen quality factor depends on three semen characteristics: ejaculate volume, sperm concentration, and number of live normal cells. Due to the insufficient number of positive males' responds to semen collection and repetitions of semen assessment (at least 5 analyzes of 3 traits for 1 ejaculate), the SQF could not be calculated for all males. Out of 140 semen collection attempts performed in each genetic group, the number of complete semen analyzes allowing the calculation of the SQF amounted only to 21 in Ry gander, and a maximum of 44 in Ki ganders. Statistical analysis showed that the average values of SQF differed significantly ($P < 0.05$) between Ka (31.2) and Ry (99.7) and Pd (89.0) ganders (Table 1). SQF of individual ejaculates in the northern group ranged from 1.9 to 141.1 (Ka), 5.1 to 345.5 (Ry) and 45.1 to 174.2 (Su), and in the group of southern ganders: 2.6–295.6 (Ki), 2.2–313.6 (Lu), and 3.1–565.5 (Pd).

DISCUSSION

Reactions to Semen Collection Procedure

Presented results showed that despite the fact that the analyzed ganders were of the same age and during the study were kept in the same environmental conditions, they differed in most of the assessed traits, both within the groups of origin, breeds and individuals of the same breed. Analyzed semen traits were at a much lower

level, compared to other poultry species, which, in fact, is characteristic for geese, especially these originated from wild ancestor *Anser anser*, from which most of the Polish goose breeds involved in the genetic resources conservation protection are derived (Smalec and Mazanowski, 1980).

In our previous experiments on ganders and drakes, we observed that if the ejaculation does not occur within 60 to 240 s after starting semen collection procedure, it is unlikely that semen will be collected at all, regardless of the method used, abdominal massage (Łukaszewicz and Kruszyński, 2003) or male stimulation by the female (Łukaszewicz et al., 2020a). Therefore, in the discussed study, the time of one male massage was limited to 120 s. Ganders of the northern breeds were less responsive to manual semen collection, several individuals did not produce a single ejaculate throughout the duration of the experiment, while all males in the southern group responded with semen ejaculation, and in one case the ejaculate was collected in each trial. The observed differences may result from the fact that northern goose varieties, unlike the southern breeds, are considered to be later maturing breeds and perhaps the males of these breeds have not reached full sexual maturity at the time of the study. However, in northern breeds there were also some males with 90 to 70% of positive reactions. Our earlier research and data described in the literature showed that the ability of males to produce semen depends on their origin (species, breed), is variable and not all males can ejaculate at a constant, even level. Within one uniform population, there are always males with very high sexual libido as well as those with very low or even zero susceptibility to the manual stimulation procedure (Kontecka et al., 1981; Gerzilov, 2004; Kowalczyk and Łukaszewicz, 2012; Kowalczyk et al., 2012; Liu et al., 2014; Zawadzka et al., 2015). Liu et al. (2008) reported that prior to the onset of the regular semen collection, ganders (semen donors) should be assessed based on their response to dorsoabdominal massage and only individuals producing ejaculates regularly should be left. Moreover, the results of Łukaszewicz et al. (2002) indicated that proper selection and training of ganders were essential for obtaining good quality semen. In our study, we intentionally selected males randomly from particular regional groups without former evaluation, so that the assessment of the evaluated traits was as objective as possible. The percentages of positively responding males varying from 36.4 to 57.1 (northern breeds) and 57.1 to 58.6 (southern breeds) seems to be low, but they were higher than in the studies by Liu et al. (2014), in which about 30% of Zi ganders (*Anser cygnoides*) and 46% of Rhin breed (*Anser anser*) ganders showed stable positive reactions, or Varga et al. (2003) who found that 30% of Hungarian ganders kept in the gene bank responded positively to the massage.

Semen Characteristics

Ejaculate Volume We documented that the quantitative and qualitative traits of gander semen are diversified and the vast majority of them depend on male origin and their individual characteristics. The ejaculate volume ranged from 0.01 mL to 0.61 mL and was similar as in the other studies conducted on ganders covered by the genetic resources protection program. Chełmońska et al. (1984) obtained from 0.25 mL to 0.30 mL ejaculate on average from Kuban ganders (descended from *Anser cygnoides*) in 2 consecutive reproductive seasons, while Opałka et al. (2008), when assessing the reproductive features of Biłgorajska ganders (Polish southern breed), collected 0.31 mL of semen volume, on average. Gumułka and Rozenboim (2015) found that the average volume of semen obtained from 2-yr-old Zatorska goose (Polish southern breed), during the following months of the reproductive season ranged from 0.17 to 0.26 mL, however, in the same experimental period as ours (February−April), ejaculate volumes were smaller, ranging from 0.21 mL to 0.24 mL. The semen volume of 1-yr-old ganders from the commercial line White Koluda amounted 0.32 mL on average, ranging from 0.15 to 0.63 mL, while in their wild ancestors Greylag (*Anser anser*) Łukaszewicz et al. (2004) obtained only 0.07 mL of semen on average (from 30 to 140 μL). A slightly higher volume, 0.21 mL on average, was obtained from Canadian goose (*Branta canadensis*) (Kowalczyk and Łukaszewicz, 2012). Similarly, low semen volumes were described by other researchers, for example, Liu et al. (2014) obtained 0.42 mL and 0.31 mL from Zi and Rhin ganders, respectively, and Liu et al. (2008) − 0.41 mL from Yangzhou ganders. Svoradová et al. (2019) were able to collect from 0.16 to 0.31 mL from Slovak White Goose, while Varga et al. (2003) from 14 Hungarian gooses obtained 258 μL on average.

Sperm Concentration The small volumes of goose ejaculates were not compensated by sperm concentration, which was also low, compared to other poultry species, and depending on breed ranged from 107.0×10^6 mL^{-1} to 1576.7×10^6 mL^{-1}. The previously cited authors who conducted research on other goose breeds covered by the protection program in Poland also noted low concentration values. In semen of Kubańska goose (*Anser cygnoides*), it averaged to 670.0 10^6mL^{-1} in the first breeding season and 580.0×10^6mL^{-1} in the second one (Chełmońska et al., 1984), in Zatorska from 37.6×10^6mL^{-1} to 331.2×10^6mL^{-1} (Gumułka and Rozenboim, 2015) and in Biłgorajska breed - 190.0×10^6mL^{-1} (Opałka et al., 2008). The concentration of sperm in the entire reproductive season of 1-yr-old White Koluda goose was 444.0×10^6mL^{-1} on average, ranging in the single male ejaculates from 35.0×10^6mL^{-1} to 1186×10^6mL^{-1} (Łukaszewicz and Kruszyński, 2003). In our later studies on the same age group, the average concentration was 307.0×10^6mL^{-1} (Łukaszewicz, 2006) and 600.0×10^6mL^{-1} (Jerysz and Łukaszewicz, 2013). In wild goose, the sperm concentration is even lower and in *Anser anser* species it averaged to 35.36×10^6mL^{-1} (Łukaszewicz et al., 2004) and 213.4×10^6mL^{-1} in *Branta canadensis* (Kowalczyk and Łukaszewicz, 2012). Different authors also confirmed the

diversified concentration of sperm. Varga et al. (2003) indicated from 0.26 to 2.25 $\times$ $10^6/\mu$L (260.0 $-$ 2250.0 $\times$ 10^6mL^{-1} in units used in our experiment) in 2-yr-old frizzled Hungarian ganders. Values similar to ours were described by Liu et al. (2014) for Zi goose (617.2 $\times$ 10^6mL^{-1}) and Rhin goose (377.0 $\times$ 10^6mL^{-1}) and Liu et al. (2008) for Yangzhou ganders (701 $\times$ 10^6mL^{-1}). Svoradová et al. (2019) reported that in the semen of Slovak White Goose, the average sperm concentration was at the level of 0.96 $-$ 1.762 $\times$ 10^9, but the authors did not specify the units or number of repetitions, the mean was calculated, and the research was carried out on three ganders aged 1 to 11 yr and "fed with wheat and oats" during the reproductive period, which could have significantly influenced the correctness of collected data.

Sperm Morphology The obtained results confirmed the essence and necessity of determining the number of live properly (normal) sperm, not only live sperm in total (including these with various deformations and damages), especially for species producing low-quality sperm, such as goose. The amount of total live sperm was at a similar level in all evaluated breeds (89.6 −97.7%), while the live normal form was much lower, depending on the breed and individual characteristics, it ranged between 15.2 and 67.9% in individual ejaculates. Similarly, Gumułka and Rozenboim (2015) found 89.4 to 94.3% of live sperm in the semen of Zatorska goose, including 41.9 to 50.0% of properly built sperm and 14.3 to 20.0% of sperm with distended heads, while Opałka et al. (2008) observed 91.17% of live sperm, 69.17% of normal sperm and 6.24% of bulb-head sperm in semen of Biłgorajska goose. The cited authors showed that, similarly to our experiment, bulb-head sperm constituted the highest percentage among the abnormal forms. In addition, in our research on ducks (Łukaszewicz et al., 2020a,c), capercaillie (Kowalczyk et al., 2012), pigeons (Klimowicz et al., 2005), and chicken broiler breeders (Łukaszewicz et al., 2020b), bulb-head sperm were observed the most frequently. Chełmońska et al. (1984) found less live sperm in total (86.2−89.0%) in Kubańska goose semen in relation to both, the northern and southern regional group, but significantly more properly formed sperm (55.7%), especially compared to Rypińska, Kielecka, and Lubelska breeds. In White Koluda gander semen, Łukaszewicz et al. (2000) observed 91.4% of live sperm including 42.9% normal and 26.5% bulb-head, similar to Jerysz and Łukaszewicz (2013). As previously mentioned, the percentage of total live sperm in semen of almost all poultry species and breeds we examined till now is high, but the amount of the most valuable−live, normal sperm is definitely smaller.

In Greylag goose ganders semen, the average proportion of total live, normal, and bulb-head sperm was 91.26, 31.17, and 35.60%, respectively (Łukaszewicz et al. 2004), and in Canada goose it was 83.2, 46.3, and 26.3%, respectively (Kowalczyk and Łukaszewicz, 2012). Compared to our data, Liu et al. (2008) stated lower (33.9%) average values of normal sperm in Yangzhou goose, the same as Liu et al. (2014) for Zi goose (40.8%) and Rhin goose (37.4%), as well as Varga et al. (2003) in Hungarian ganders.

Sperm Quality Factor The great variety of techniques for semen quality evaluation and very large diversity in individual male characteristics, not only between species and breeds, but also between individuals of the same genetic group, make their assessment and comparison difficult. The SQF proposed by the author of this experiment (Łukaszewicz and Kruszyński, 2003), covering 3 basic (and easy to assess) semen characteristics seems to be a good semen quality indicator, allowing for a reliable assessment of male suitability for artificial insemination, and has already been successfully applied by other researchers (Liu et al., 2008; Kowalczyk and Łukaszewicz, 2012; Gumułka and Rozenboim, 2015). In the presented study, in all assessed breeds, slightly more than half of the collected ejaculates were suitable for determining the SQF, which indicates a very low percentage of positive reaction to semen collection procedure, followed by ejaculation of good quality semen. In the available articles, only Gumułka and Rozenboim (2015) assessed the SQF of gander semen covered by the genetic resources protection program−−Zatorska geese, and showed that in May this factor amounted 37.3 per one male, while in June it dropped to 6.4. A comparable SQF value (39.6) was characteristic for White Koluda ganders (line selected from White Italian goose) in the first year of reproduction (Łukaszewicz and Kruszyński, 2003). Compared to the presented results, similar or higher average SQF values were found for Yangzhou goose (84.0; Liu et al., 2008), Zi and Rhin goose (130.4 and 51.3, respectively, Liu et al., 2014). Significantly lower SQF was found for Greylag − 1.06 (Łukaszewicz et al., 2004) and for Canada ganders (20.3, Kowalczyk and Łukaszewicz, 2012). It can be assumed that in wild geese characterized by monogamy, the number of sperm necessary for ovum fertilization is lower than in the domesticated geese who lays more eggs and for a longer period, therefore such a low SQF could be observed.

The SQF determines the number of live normal sperm in one ejaculate, and assuming that the reproductive ability of males depends on the amount of this cell, and their number necessary for successful fertilization depends on the particular species (Łukaszewicz, 2002; Kowalczyk and Łukaszewicz, 2012), this indicator shows how many insemination doses can be created from one ejaculate. In the discussed study, the highest index among the northern ganders (139.6) was recorded for the Rypińska gander with 92.9% of positive reactions, and the lowest (10.1) for the gander with 64.3% positive reactions. Among the southern varieties, the highest SQF value (253.6; 92.9% of reactions) was observed in Podkarpacka gander, and the lowest (25.2 and 50.0% positive reactions) in Kielecka. Analyzing the average values for northern and southern breeds, in the former one 45.9% of positive responses and SQF at the level of 61.9 were obtained, while in the southern breeds−−57.6 and 72.7%, respectively. This suggests that if a male

responds poorly to dorsoabdominal massage, and within 120 s ejaculation will not occur, it is very likely that the quality of the semen will also not be high and such male should not be left as a semen donor.

Creating the genetic reserves ex situ in vivo is certainly a valuable form of species protection and preservation of valuable genes of species and breeds not changed by strictly directed genetic selection, however, attention should be paid to the quality of semen and to elimination of males that are poor in this respect, in order not to lead to an excessive weakening of the reproductive capacity of the flocks covered by the genetic resources protection program.

DISCLOSURES

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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REFERENCES

Bakst, M. R., G. Wishart, and J. P. Brillard. 1994. Oviducal sperm selection, transport, and storage in poultry. Poult. Sci. Rev. 5:117–143.

B.D. Scherf (ed.). 2000. *Watch World List for Domestic Animal Diversity.* (3rd ed.). Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org/3/a-x8750e.pdf.

Blesbois, E., I. Grasseau, F. Seigneurin, S. Mignon-Grasteau, M. S. Saint Jalme, and M. M. Mialon-Richard. 2008. Predictors of success of semen cryopreservation in chickens. Theriogenology 69:252–261.

Blesbois, E., F. Seigneurin, I. Grasseau, C. Limouzin, J. Besnard, D. Gourichon, G. Coquerelle, P. Rault, and M. Tixier-Boichard. 2007. Semen cryopreservation for ex situ management of genetic diversity in chicken: creation of the French Avian Cryobank. Poult. Sci. 86:555–564.

Bódi, L., J. Szalay, and T. N. Phuong. 2019. Gene conservation practice and production of old Hungarian goose breeds. Sci. J. Tra Vinh University 134:32–36.

Chełmońska, B., E. Koch, and M. Chrzanowska. 1984. The fertilizing ability of frozen gander semen depending on the frequency of geese insemination. Instyt. Zootechniki. Wyniki Prac. Badawczych Zakładu Hodowli Drobiu 10:191–197 in Polish, abstract: English, Deutsch, Русский язык.

Dobrzański, J., J. Calik, J. Krawczyk, and T. Szwaczkowski. 2019. Conservation of goose genetic resources in Poland - past and present status. Worlds Poult. Sci. J. 75:387–400.

Gerzilov, V. 2004. A study on the semen characteristic and sexual activity of one-and two-year old Muscovy Drakes. Bulg. J. Agric. Sci. 10:143–148.

Graczyk, M., K. Andres, E. Kapkowska, and T. Szwaczkowski. 2018. Genetic evaluation of reproductive potential in the Zatorska goose under a conservation program. Anim. Sci. J. 89:752–759.

Gumułka, M., and I. Rozenboim. 2015. Breeding period-associated changes in semen quality, concentrations of LH, PRL, gonadal steroid and thyroid hormones in domestic goose ganders (*Anser anser f. domesticus*). Anim. Reprod. Sci. 154:166–175.

Jerysz, A., and E. Łukaszewicz. 2013. Effect of dietary selenium and vitamin E on ganders' response to semen collection and ejaculate characteristics. Biol. Trace Elem. Res. 153:196–204.

Klimowicz, M., E. Łukaszewicz, and A. Dubiel. 2005. Effect of collection frequency on quantitative and qualitative characteristics of pigeon (*Columba livia*) semen. Br. Poult. Sci. 46:361–365.

Kontecka, H., E. Cegielska, and A. Skrzydlewski. 1981. The examinations of morphological changes of Muscovy drakes spermatozoa in the first year of their reproductive period. Rocz. Akad. Roln. Poznaniu107–113 CXXXin Polish, summary in English.

Kowalczyk, A., and E. Łukaszewicz. 2012. The possibility of obtaining intergeneric hybrids via White Koluda (*Anser anser* L.) goose insemination with fresh and frozen-thawed Canada goose (*Branta canadensis* L.) gander semen. Theriogenology 77:507–513.

Kowalczyk, A., E. Łukaszewicz, and Z. Rzońca. 2012. Successful preservation of capercaillie (*Tetrao urogallus* L.) semen in liquid and frozen states. Theriogenology 77:899–907.

Krawczyk, J., J. Obrzut, and R. Zwierzyński. 2014. Analiza wyników użytkowości i efektywnej wielkości populacji krajowych gęsi czterech odmian południowych. Rocz. Nauk. Zoot. 41:107–118 (in Polish, summary in English).

Książkiewicz, J. 2007. Historical overview of the creation of conservation goose breeds owned by the National Research Institute - PIB. Wiadomości. Zootech. XLV 3:77–81.

Liu, S. J., J. L. Li, and H. Li. 2014. A diagnostic method for gander selection in Zi geese (*Anser cygnoides* L.) and Rhin geese (*Anser anser* L.) using massage reaction and semen quality. Anim. Prod. Sci. 54:569–573.

Liu, S. J., J. X. Zheng, and N. Yang. 2008. Semen quality factor as an indicator of fertilizing ability for geese. Poult. Sci. 87:155–159.

Łukaszewicz, E. 2002. An effective method for freezing White Italian gander semen. Theriogenology. 58:19–27.

Łukaszewicz, E. 2006. Characteristics of fresh gander semen and its susceptibility to cryopreservation in six generations derived from geese inseminated with frozen-thawed semen. CryoLetters 27:51–58.

Łukaszewicz, E., M. Chrzanowska, A. Jerysz, and B. Chełmońska. 2004. Attempts on freezing the greylag (*Anser anser*) gander semen. Anim. Reprod. Sci. 80:163–173.

Łukaszewicz, E., H. Furuta, Y.-M. Xi, and N. Fujihara. 2000. Comparative study on semen quality of one- and two-year-old ganders during the entire reproductive season. Asian J. Androl. 2:139–142.

Łukaszewicz, E., A. Jerysz, and B. Chełmońska. 2020a. Effect of semen extenders and storage time on quality of Muscovy duck (*Cairina moschata*) drake semen during the entire reproductive season. Reprod. Dom. Anim. 55:943–950.

Łukaszewicz, E., A. Jerysz, and A. Kowalczyk. 2020b. Effect of semen extenders on viability of ISA Brown and Hubbard Flex roosters' sperm stored for 24 h. Poult. Sci. 99:2766–2774.

Łukaszewicz, E., A. Jerysz, and A. Kowalczyk. 2020c. Reproductive season and male effect on quantitative and qualitative traits of individually collected Muscovy duck (*Cairina moschata*) semen. Reprod. Dom. Anim. 55:1735–1746.

Łukaszewicz, E., A. Kowalczyk, and Z. Rzońca. 2011. Characteristics of fresh semen of captive-bred capercaillie *Tetrao urogallus* L. Zoo Biol 30:672–680.

Łukaszewicz, E., and W. Kruszyński. 2003. Evaluation of fresh and `frozen-thawed semen of individual ganders by assessment of spermatozoa motility and morphology. Theriogenology 59:1627–1640.

Mazanowski, A., M. Adamski, T. Kisiel, and M. Urbanowski. 2006a. Porównanie cech mięsnych i reprodukcyjnych krajowych odmian gęsi południowych i północnych. Rocz. Nauk. Zoot. 33:105–123 (in Polish, summary in English).

Mazanowski, A., Z. Bernacki, M. Adamski, and T. Kisiel. 2006b. Analysis of time trends for reproductive and meat traits in

randomly mated conservation flocks of northern variety geese. Ann. Anim. Sci. 6:59–74.

Mazanowski, A., T. Kisiel, and M. Adamski. 2005. Evaluation of some regional varieties of geese for reproductive traits, egg structure and egg chemical composition. Ann. Anim. Sci. 5:67–83.

Opałka, M., J. Kugla-Owczarska, B. Kamińska, H. Puchajda-Skowrońska, W. Hryniewicka, and L. Dusza. 2008. Effects of dietary meals containing different levels of phytoestrogens on reproductive function in Bilgoraj ganders. Acta Vet. Hung. 56:379–391.

Saint Jalme, M., R. Lecoq, F. Seigneurin, E. Blesbois, and E. Plouzeau. 2003. Cryopreservation of semen from endangered pheasants: the first step towards a cryobank for endangered avian species. Theriogenology 59:875–888.

Smalec, E., and A. Mazanowski. 1980. The goose gene pool. A characteristic of native varieties. In *Proc. 6 Europ. Poult. Conf* (pp. 202−209)202–209.

Svoradová, A., A. Baláži, J. Vašíček, C. Hrnčár, and P. Chrenek. 2019. Quality evaluation of fresh gander semen of Slovak white goose by casa and flow cytometry: short communication. Slovak J. Anim. Sci. 52:90–94.

Varga, A., J. Barna, and A. Almasi. 2003. Sperm analysis and sexual characteristics of frizzled Hungarian ganders (Preliminary study). Hung. J. Anim. Prod. 52:167–172.

Zawadzka, J., E. Łukaszewicz, and A. Kowalczyk. 2015. Comparative semen analysis of two Polish duck strains from a conservation programme. Europ. Poult. Sci. 79:1–9.