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Study of Semen Quality, Reproductive Hormone Levels, and Lipid Levels in Men From Arkhangelsk, a City in North of European Russia

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Abstract

Male populations in the European North of Russia have not previously been investigated for semen quality. The aim of this study was to evaluate semen parameters, reproductive hormone levels, and lipid levels in volunteers from the general urban population of the European North of Russia, to compare the data published for men from the neighboring Northern or Eastern European countries, and to evaluate associations between sperm quality and serum hormonal and lipid levels. Ninety-nine volunteers aged 23–63 years residing in the city of Archangelsk were enrolled in the study. All men had blood samples drawn and completed a questionnaire concerning their health status and lifestyle; 90 men delivered semen samples. The medians for semen volume, sperm concentration, progressive motility, and normal morphology were 3.0 ml, 42.12 million/ml, 43.8%, and 6.5%, respectively. Sperm parameters below normal threshold values were found in 38.9% of participants. It seems that the sperm quality in our study group was slightly worse than in men from Finland, Norway, Sweden, or Estonia, but very similar to that in men from Denmark or Poland. The significant negative correlations of luteinizing hormone levels and positive correlations of inhibin B levels with sperm concentration and progressive motility were revealed. Higher levels of luteinizing hormone and lower levels of inhibin B were found in participants with impaired compared to normal sperm quality. No reliable links were found between serum total cholesterol, triglyceride, high and low-density lipoprotein cholesterol, and semen parameters.

Keywords

semen quality, reproductive hormones, serum lipids, general population, European North of Russia

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Over the past three decades, a number of studies showed a time-related decrease of semen quality as well as an increase of male infertility and incidence of some diseases associated with the male reproductive system (Basnet et al., 2016; Giwercman et al., 1993; Jiang et al., 2014; Levine et al., 2017; Mínguez-Alarcón et al., 2018; Mishra et al., 2018; Skakkebaek et al., 2016; Swan et al., 2000). Abundant literature has identified considerable regional differences in semen parameters between and within countries (Erenpreiss et al., 2017; Halling et al., 2013; Jørgensen et al., 2002; Kamieniczna et al., 2015; Paasch et al., 2008; Punab et al., 2002; Swan et al., 2003; Zou et al., 2011). The reasons of temporal or geographic differences in semen quality remain poorly understood; however, different climatic conditions, environmental toxicants, lifestyle, and genetic backgrounds are considered as important contributors to male reproductive health (Skakkebaek et al., 2016).

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There is no information about the sperm parameters in residents of the circumpolar region of Russia, and there are very little data on sperm quality in other ethnic populations living in this area. The circumpolar populations are subject to prolonged exposure to adverse climatic factors (low air temperature, marked seasonality, a peculiar photoperiod with very low daylight hours in winter and elevated ones in summer) and environmental contaminants (persistent organochlorine pollutants [POPs]), and are characterized by specific lifestyle habits including traditional lipid-rich marine food.

The length of the daylight period does not cause substantial changes in sperm concentration and motility, and has a slight impact on follicle-stimulating hormone (FSH) and inhibin B levels as was demonstrated in a study on Norwegian men living north and south of the Arctic Circle (Malm et al., 2004). International comparisons showed that semen quality for men from the Inuit population of Greenland and the Faroese Islands was low compared to men from other European countries due to high exposure to POPs (Halling et al., 2013; Toft et al., 2004). The Arctic region was contaminated with POPs, and high concentrations of these compounds were found in blood samples of the local Arctic populations (Hung et al., 2016). Environmental exposure to POPs reduced sperm motility in Greenland and the Swedish fishermen populations (Toft et al., 2006), and altered the reproductive hormone profile of Norwegian men, but did not affect semen quality (Haugen et al., 2011). In the Inuit population of Greenland, the sperm concentration and morphology were not impaired by increasing serum levels of perfluorinated compounds; however, sperm motility was inversely related to concentration of pollutants (Toft et al., 2012). Delayed conception rate related to serum POP levels was observed in Inuit people of Greenland where fecundability was reduced by 30% among the high-level-exposed groups (Bonde et al., 2008). A cross-sectional study involving males from Greenland, Sweden, Poland, and Ukraine has shown a strong increasing DNA fragmentation index with increasing serum POP levels among European but not Inuit men (Spanò et al., 2005). Bonde et al. (2008) and Toft (2014) reviewed in detail the adverse effects of environmental exposure to POPs on human reproductive health both in the Arctic and European populations.

The conventional analysis of sperm quality according to the WHO guidelines remains "gold standard" for evaluating male fertility in a clinical practice and epidemiological studies. Additionally, much attention is paid to reproductive hormones as potential markers of spermatogenesis, including testosterone, FSH, luteinizing hormone (LH), and inhibin B, which are crucial for initiation and maintenance of spermatogenesis (Iliadou et al., 2015; Ramaswamy & Weinbauer, 2014; Smith & Walker, 2014). Determining the relationships between levels of testosterone, LH, FSH, inhibin B, and sperm parameters is an important step toward a better understanding of the basic mechanisms by which hormones control normal or impaired spermatogenesis, and ultimately to a better understanding of fertility regulation. The relationship between the levels of reproductive hormones and sperm parameters has been investigated mainly in fertile or infertile men, while studies on men from the general population have been very rare (Andersson et al., 2004a, 2004b; Barbotin et al., 2015; Jørgensen et al., 2016). The study of the relationship between reproductive hormone levels and sperm parameters is particularly relevant for men living in the circumpolar North, since information about the features of hormonal regulation of spermatogenesis in such populations remains unexplored.

Recent experimental data have demonstrated the importance of lipid metabolism in the control of testicular function and male fertility (Shi et al., 2018; Whitfield et al., 2015). Lipids play an important role in the structural and functional activity of sperm especially in post-testicular sperm maturation and capacitation. Mammalian spermatozoa expend energy, generated as intracellular adenosine triphosphate (ATP), largely on motility to attain access to their conspecific egg. Lipids also acts as precursors for the biosynthesis of sex steroid hormones, which in turn, regulate reproductive functions. Data regarding the relation of serum lipid profile to sperm parameters are conflicting in terms of the nature and magnitude of the effect. Although some studies have found a significant negative relationship between serum lipid levels and sperm parameters (Ergün et al., 2007), other studies have not found such an association (Eisenberg et al., 2015; Lu et al., 2016). Positive correlations between serum levels of triglyceride and very low-density lipoprotein and sperm concentration, or between serum levels of low-density lipoprotein and cholesterol and sperm progressive motility were demonstrated in a large-scale general male population (Liu et al., 2017). Thus, it is unclear whether the lipid spectrum will reflect the seminal parameters in men from our study population. Given that the circumpolar populations of indigenous and non-indigenous people are characterized by specific metabolic adaptations maintaining thermogenesis in cold conditions such as elevated basal metabolic rate and increased fatty acid metabolism (Leonard et al., 2002), it can be assumed that metabolic adaptations may be associated with changes in sperm quality and hormonal patterns in men. To obtain an answer to this question, serum lipid levels were determined and the correlations with semen parameters were evaluated in this study.

The main objectives of this study were: (a) to investigate semen parameters, serum reproductive hormone, and lipid levels in men from the general population residing in

the European North of Russia; (b) to evaluate associations between semen parameters, serum reproductive hormone, and lipid levels; and (c) to compare the semen results with data from neighboring European populations.

Methods

Study Population

The investigation took place at the Federal Center for Integrated Arctic Research, the Russian Academy of Sciences, Arkhangelsk, Russia. The city of Arkhangelsk is a big industrial center in European North of Russia located within the circumpolar area. This area is characterized by maritime moderately cool climate and considerable seasonal differences in sunlight length. Male volunteers aged 23–63 years (n = 99) from the general population of Arkhangelsk were enrolled in the study. The study group consisted predominantly of ethnic Russians. Men were informed about the study through advertisement on the Internet and TV (All-Russian State Television and Radio Broadcasting Company "Pomorie"). The advertisement provided comprehensive information about the purpose of the investigation. In particular, it was reported that the investigation provides an opportunity for assessing the semen quality and endocrine functions of the male population of the city of Arkhangelsk and for participants to determine the state of their reproductive health and endocrine status. Any man over the age of 17 who wanted to know his reproductive health and endocrine status was invited to participate in the study. Complete anonymity of the survey was guaranteed. A candidate volunteer had to call the institute and make an appointment for the investigation. In this telephone conversation, each participant was instructed to abstain from ejaculation and alcohol consumption for 2–3 days prior to producing the semen sample. Abstinence from alcohol consumption was recommended to avoid possible consequences of the direct and sharp alcohol effect on reproductive hormone levels (Emanuele & Emanuele, 2001). Additional exclusion criteria for participation were general or chronic diseases in an acute phase, genital tract infections, or other acute infections with appropriate medication. A total of 158 men contacted by phone, 99 of these visited the Center for examination, and 90 men delivered sperm samples; hence, the participation rate was 57%.

All participants gave informed consent for participation, and filled out a standardized questionnaire including information on current age, place of birth, nationality, parents and grandparents information, family status, frequency of alcohol consumption and tobacco smoking, profession, and previous or current urological diseases. Previous urogenital disorders specified in the questionnaires included cryptorchidism, urogenital infections, prostatitis, and varicocelectomy. Current urogenital disorders were diagnosed in the examination, irrespective of previous self-reported information, and included clinical varicocele grade II, prostatitis, testicular cysts, hydrocele, and hypospadias.

Physical Examination

Each participant was examined by the same experienced andrologist on the day of delivery of his blood and semen sample, and the results were recorded in a standard protocol. Body weight (kg) was measured with participants wearing light clothing using an electronic scale. Height (cm) was measured using a standard stadiometer. Waist and hip circumference (cm) was measured. Waist to hip ratio (WHR) was calculated as the ratio of waist circumference to hip circumference, and waist to height ratio (WHtR) was calculated as the ratio of waist circumference to height. Body mass index (BMI) was calculated as kg/m^2. Secondary sexual characteristics; possible presence of varicocele, hydrocele, or hypospadias; location of the testis in the scrotum; and testicular size were examined to establish a preliminary urological diagnosis. Testicular size was determined using a Prader orchidometer (made of wood, "Valkiria," Russia) and expressed in ml. Mean testicular size was chosen; preliminary analyses showed significant correlation among left, right, and mean testicular volume (data not shown). Age was calculated as difference between date of attendance in study and self-reported date of birth.

Blood and Sperm Collection

In this study, we describe ejaculate volume, sperm concentration, progressive sperm motility and normal morphology, serum concentrations of FSH, LH, inhibin B, total and free testosterone, estradiol, sex hormone-binding globulin and serum levels of triglycerides, total cholesterol, and high- and low-density lipoprotein cholesterol in men living in the city of Arkhangelsk located within the circumpolar area. For this purpose, each participant provided one blood and sperm sample on the same day. Fasting blood samples from the cubital vein were drawn in the morning between 9–11 hr, before the semen samples were collected. Blood samples were centrifuged; serum was stored at −40° C until analysis. Semen samples were obtained by masturbation into disposable sterile plastic containers in special private room close to the laboratory. All men were encouraged to follow the abstinence period of 2–3 days according to the WHO laboratory manual for the Examination and processing of human semen (World Health Organization, 2010), but in each case, the actual length of the sexual abstinence period was recorded. Ejaculation abstinence period was calculated as difference between time of current ejaculation and self-reported time of previous ejaculation.

Semen Analysis

Semen samples were analyzed for semen volume (ml), sperm concentration ($\times 10^6$/ml), normal morphology (percentage) according to the WHO laboratory manual for the Examination and processing of human semen (World Health Organization, 2010), but sperm progressive motility was determined by the automatic sperm analyzer SFA-500 ("Biola," Russia). Ejaculate volume was estimated by weighing the collection container and subtracting the weight of the preweighed empty container assuming that 1 ml ejaculate weighs 1 g. Semen samples were liquefied in an electro thermostatic water bath at 37 °C not more than 1 hr. Sperm concentration was determined with a Goryaev's hemocytometer under light microscope (magnification ×400). The Goryaev's hemocytometer is similar to the improved Neubauer hemocytometer recommended by the WHO laboratory manual (World Health Organization, 2010), and it is widely used in reproductive centers in Russia. To determine sperm concentration, 100 μL of well-mixed native ejaculate was dissolved in 400 μL of the mixture (5% $NaHCO_3$; 0.35% formaldehyde; 0.025% trypan blue in distilled water). Preliminary staining of spermatozoa with trypan blue makes it possible to clearly distinguish sperm heads under light microscopy. Spermatozoa were counted independently in two chambers of the hemocytometer using one dilution (at least 200 spermatozoa were counted per replicate) and the mean value was calculated. Total sperm count was then calculated by multiplying the individual's sperm concentration by the ejaculate volume.

As mentioned above, sperm progressive motility (WHO motility classes A + B) was determined using the automatic sperm analyzer SFA-500 ("Biola," Russia). The evaluation principle was based on a measurement of fluctuations of optical density in native ejaculate as a result of sperm movement through optical channel illuminated by a laser beam. Optical fluctuations were registered by a photodetector; the number of sperm with rapid progressive motility (velocity ≥25 μm/s; WHO class A) and slow progressive motility (velocity 5–25 μm/s; WHO class B) was calculated by a special software. A well-mixed aliquot of native ejaculate (50 μL) is placed in a special glass chamber where the temperature is maintained at 37º C. The sperm motility measurements were carried out three times for each sample and the average value was calculated.

For sperm morphology analysis, ejaculate smears from each participant were prepared on two glass slides, air-dried and fixed in methanol before staining. Diff-Quick kits ("Abris plus," Russia) were used for staining the slides according to the manufacturer manual. Two hundred spermatozoa were examined for morphology with the optical microscope (Axio Skop.A1, "Carl Zeiss," Germany) at ×1000 magnification with oil-immersion according to the WHO manual (World Health Organization, 2010). Sperm morphology evaluations were done in duplicates in random and blinded order by a single trained junior researcher, one of the authors (M.K.). Here, we report the percentage of sperm scored as morphologically normal (%). Although the assessment of multiple sperm defects was not necessary for routine semen analysis, the teratozoospermia index (TZI) was also calculated for each sample (Barratt et al., 2011; Mortimer & Menkveld, 2001). The TZI is the average number of defects per abnormal spermatozoon (Mortimer & Menkveld, 2001). To determine the TZI, the total number of defects determined was divided by the number of abnormal spermatozoa.

Hormone and Lipid Assay

Serum hormone concentrations (total testosterone, T; FSH; LH; estradiol, E_2; inhibin B, InhB; free testosterone, fT) and sex hormone-binding globulin, SHBG were determined in duplicate by enzyme immunoassay. Commercially available kits "Steroid IFA-Testosterone-01," "Gonadotropin IFA-LH," "Gonadotropin IFA-FSH" (Alkor Bio, Russia), "Estradiol-IFA" (Xema Medica, Russia), "Inhibin B Gen II ELISA" (Beckman Coulter, USA), "SHBG ELISA," and "Free Testosterone ELISA" (DRG International, USA) were used according to the manufacturer manuals. The intra- and interassay coefficients of variation were as follows: T $<$ 8.0%; E_2 $<$ 8.0%; FSH $<$ 8.0%; LH $<$ 8.0%; InhB $\leq$ 6.8%; fT $\leq$ 8.9% and $\leq$ 12.4, respectively; SHBG $\leq$ 3.0 and $\leq$ 5.4%, respectively. The sensitivities for T, E_2, FSH, LH, InhB, fT, and SHBG were 0.2 nmol/L, 0.025 nmol/L, 0.25 mIU/ml, 0.25 mIU/ml, 2.6 pg/ml, 0.04 pg/ml, and 0.77 nmol/L, respectively.

Serum concentrations of triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) were determined in duplicate by enzymatic colorimetric methods using commercially available kits ("Vector Best," Russia). The upper limits of evaluated concentrations for TG, TC, and HDL-C were 11.4 mmol/L, 27 mmol/L, and 3.0 mmol/L, respectively. Serum concentration of low-density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald formula (Friedewald et al., 1972).

Statistical Analysis

The statistical analysis of the data obtained was performed using the statistical package "STATISTICA" (version 8.0). The results are presented as mean (SD) as well as

median with 5th and 95th percentiles. The Kolmogorov–Smirnov test for normality was used. Sperm concentration, total sperm count, FSH, T, SHBG, and TG levels were not normally distributed, and they were best normalized by log transformation to correct for skewed distribution of residuals. Other parameters were close to that of a normal distribution and used untransformed. Descriptive statistics is presented using untransformed data. Spearman correlation coefficients were used to determine correlations among semen, anthropometric, hormonal, and lipid parameters. Differences in anthropometric, semen, hormonal, and lipid variables between groups with different semen quality were tested by analysis of covariance (ANCOVA); semen, hormonal and lipid variables were adjusted for a period of abstinence and age. A p value $<$ .05 was regarded as statistically significant.

Results

Anthropometrical and SocioDemographic Characteristics

The ethnic composition of the study group was as follows: 88 (88.9%) were Slavs (Russians, Belarusians, and Ukrainians); 10 (10.1%) were descendants of a mixed marriage between Russians and Tatars, or Chuvash, or Komi, or Germans; and one (0.1%) was a representative of the Balkar ethnic group. Most participants (83, 84.9%) were born in the city of Arkhangelsk or in the Arkhangelsk region; others were residents of this area for 5–42 years (mean 27.3 ± 3.5 years). According to the questionnaire information, 56 (56.6%) participants were in professions not related to physical labor, while 40 (40.4%) were engaged in physical labor or a combination of physical and mental labor; data were missing for two men (3.0%).

The anthropometrical and sociodemographic characteristics of the study group are summarized in Table 1. Participants were 21–63 years old (median age 35 years). The entire group was characterized by increased median BMI in comparison with the accepted threshold for normal body mass index. Of the participants in our study group, only 36 (36.4%) had normal BMI, 44 (44.4%) were overweight, and 19 (19.2%) suffered from obesity. There were no men categorized as underweight (BMI $<$ 18.5 kg/m^2). The median waist circumference was 90.0 cm, but 41 (41.4%) participants had higher than normal waist circumference ($<$94 cm). According to WHtR categories (Ashwell et al., 2012), median WHtR for the entire group was characterized by increased value, and 64 (64.7%) men had WHtR higher than normal value ($<$0.5).

Age was positively correlated with waist circumference, WHR, and WHtR (r = .288, p = .004; r = .353, p < .001; r = .381, p < .001, respectively). There was no correlation between age and BMI.

Table 1. Anthropometric Parameters and Self-Reported Information of the Participants From the City of Archangelsk (n = 99).

Physical appearance	Mean (*SD*)	Median (5–95)
Age, years	37.8 (10.9)	35.0 (23.0, 58.0)
Height, cm	177.2 (6.7)	177.0 (165.0, 187.5)
Weight, kg	84.4 (13.7)	85.0 (63.0, 108.0)
Waist circumference, cm	92.1 (9.4)	90.0 (78.0, 107.0)
Hip circumference, cm	103.7 (5.7)	104.0 (94.0, 113.0)
WHR	0.89 (0.06)	0.88 (0.79, 0.98)
WHtR	0.52 (0.05)	0.51 (0.44, 0.62)
BMI, kg/m^2	26.9 (4.0)	26.5 (21.1, 34.4)
Mean of left and right testis volume, ml	25.3 (4.5)	25.0 (17.5, 30.0)
Sexual abstinence, days	3.9 (3.3)	3.0 (2.0, 14.0)
Lifestyle	**Number**	**% of the study group**
Married men	71	71.7
Men having children	52	52.5
Cigarette smokers	39	39.4
Drinkers	73	73.7
Previous urogenital disorders	34	34.3
Current urogenital disorders	14	14.1

Note. SD = standard deviation; (5–95) = 5th–95th percentile; WHR = waist to hip ratio; WHtR = waist to height ratio; BMI = body mass index. Data are missing on testis volume for one man and on waist and hip circumference for two men.

Of the 71 married men, only 52 (73.2%) had children (mean 1.7 ± 0.1). Among the participants, 39 (39.4%) reported that they were smokers (mean 16.9 ± 1.3 cigarettes per day), but most (87.2%) smoked 10 or more cigarettes per day. More than two-thirds of the participants reported alcohol consumption (mean 1.1 ± 0.1 times per week) of beer, wine, or vodka. About one-third of the participants reported having previously suffered urogenital diseases; however, the presence of urogenital disorders was detected at the physical examination in only 14.1%.

Semen Parameters

Out of 99 participants, nine men refused to donate ejaculate, so only 90 semen samples were collected. Semen parameters are summarized in Table 2. Briefly, the median semen volume was 3.0 ml; sperm concentration 42.12 × 10^6/ml; total sperm count 128.03 × 10^6; progressive motility 43.8%; and normal sperm morphology 6.50%. The absence of spermatozoa observed in whole ejaculate was considered as azoospermia, and the sperm

Table 2. Semen, Hormonal, and Lipid Parameters of Participants From the City of Archangelsk ($n = 99$).

Variable	Mean (*SD*)	Median (5–95)
Semen volume, ml	3.3 (1.8)	3.0 (1.1, 5.9)
Sperm concentration, $\times 10^6$/ml	55.81 (45.77)	42.12 (9.00, 157.08)
Total sperm count/ejaculate, $\times 10^6$	175.09 (153.21)	128.03 (17.82, 467.23)
Progressive motility, %	45.0 (26.5)	43.8 (5.7, 89.8)
Normal morphology, %	6.95 (2.98)	6.50 (2.00, 12.00)
TZI	1.49 (0.09)	1.48 (1.34, 1.62)
LH, mIU/ml	3.44 (1.44)	3.14 (1.61, 7.07)
FSH, mIU/ml	4.11 (2.84)	3.54 (1.29, 9.02)
Total testosterone, nmol/L	16.79 (7.35)	14.66 (8.37, 31.96)
Free testosterone, pg/ml	12.39 (8.03)	11.40 (3.60, 23.10)
Estradiol, nmol/L	0.184 (0.048)	0.177 (0.125, 0.297)
Inhibin B, pg/ml	199.85 (51.82)	200.01 (101.03, 295.66)
SHBG, nmol/L	51.03 (31.60)	41.90 (19.50, 126.90)
TG, mmol/L	1.33 (0.71)	1.10 (0.41, 2.70)
TC, mmol/L	4.43 (1.00)	4.27 (2.89, 6.56)
HDL-C, mmol/L	1.17 (0.39)	1.06 (0.65, 1.95)
LDL-C, mmol/L	2.67 (1.00)	2.61 (1.21, 4.89)

Note. SD = standard deviation; (5–95) = 5th–95th percentile; TZI = teratozoospermia index; LH = luteinizing hormone; FSH = follicle-stimulating hormone; SHBG = sex hormone-binding globulin; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol. Results are based on raw data. Of 99 participants, semen parameters were determined only in 90. Data are missing on sperm progressive motility and normal morphology for two men (azoospermia or heavy oligozoospermia) and on HDL-C and LDL-C for one man.

concentration and total sperm count per ejaculate less than the reference values (15 $\times 10^6$ sperm/ml and 39 $\times 10^6$ sperm/ejaculate) as oligozoospermia. Percentage of motile spermatozoa with progressive motility (class A + B) less than 32% was considered as asthenozoospermia, and that of morphologically normal spermatozoa less than 4.0% as teratozoospermia. Reference values for oligozoospermia, asthenozoospermia, and teratozoospermia were taken in accordance with the WHO guidelines (World Health Organization, 2010).

Of 90 semen samples, only one was azoospermic; it was centrifuged, and no sperm were detected in the centrifuged pellet. Oligozoopermia was revealed in eight (8.9%) participants including a man with a very low sperm concentration (<1 million/ml), asthenozoospermia in 30 men (33.3%), teratozoospermia in 12 men (13.3%). Thirty-five (38.9%) participants had at least one of the semen parameters (sperm concentration, motility or normal morphology) below the reference limits (World Health Organization, 2010). Overall, 55 (61.1%) participants had normal semen analysis according to WHO guidelines (World Health Organization, 2010).

According to the WHO laboratory manual (2010), the TZI (the mean number of anomalies per abnormal sperm) is based on four abnormality classes: one each for head, midpiece, tail, and cytoplasmic drops but does not include relevant reference values (Barratt et al., 2011). In our study, the median TZI was equal to 1.48, which can be interpreted as falling within approximately normal range, taking into account the TZI values when comparing a fertile (TZI = 1.46) and subfertile population (TZI = 1.64; Menkveld et al., 2001).

Significant upward trends in semen volume and total sperm count were observed with increasing abstinence period: positive correlations were observed between duration of sexual abstinence and semen volume, and total sperm count ($r = .225$, $p = .03$; $r = .266$, $p = .01$, respectively). Other semen variables (sperm concentration, progressive motility, normal morphology) were not affected by duration of sexual abstinence. Sperm concentration was positively correlated with progressive motility and normal morphology ($r = .842$, $p < .0001$; $r = .630$, $p < .0001$, respectively). Total sperm count was positively related to sperm progressive motility and normal morphology ($r = .730$, $p < .0001$; $r = .496$, $p < .0001$, respectively). There was a significant positive correlation between progressive motility and normal morphology ($r = .740$, $p < .0001$).

Hormonal and Metabolic Parameters

Data on hormonal and lipid variables of the participants are presented in Table 2. The median concentrations of LH, FSH, T, E_2, inhB, and SHBG were within normal ranges. Of the 99 participants, 25 (25.3%) had T levels below normal reference value according to recommended

12.1 nmol/L as a lower limit (Lunenfeld et al., 2015). Only three participants with abnormal low T levels had a sperm concentration lower than 15×10^6/ml.

In this study, measurements of serum TG, TC, and HDL-C levels were performed and LDL-C level was calculated (Table 2). There were 30 (30.3%), 20 (20.2%), 44 (44.9%), and 23 (23.5%) men with serum TG, TC, HDL-C, and LDL-C levels out of their normal reference values, respectively (below 1.70 mmol/L for TG; below 5.2 mmol/L for TC; above 1.03 mmol/L for HDL-C; below 3.36 mmol/L for LDL-C). Of the entire study group, 45 (45.5%) participants had dyslipidemia.

Relationships Between Age, BMI, and Serum Hormonal and Lipid Concentrations

Age was positively related to FSH and TC concentrations ($r = .409$, $p = .000$; $r = .252$, $p = .012$, respectively) and negatively related to TT, fT, and inhB concentrations ($r = -0.205$, $p = .042$; $r = -0.451$, $p = .000$; $r = -0.359$, $p = .000$, respectively). The serum concentrations of other hormones and metabolites were not correlated with age. Significant negative relationships were observed between BMI and T concentrations ($r = -0.398$, $p = .000$) as well as between BMI and E_2 and SHBG concentrations ($r = -0.243$, $p = .016$; $r = -0.395$, $p = .000$, respectively). BMI showed significant positive correlations with concentrations of TG, TC, and LDL-C ($r = .428$, $p = .000$; $r = .256$, $p = .011$; $r = .229$, $p = .023$, respectively), but significant negative correlation with concentration of HDL-C ($r = -0.257$, $p = .011$).

Statistically significant positive relationships were found between LH and FSH, T, and SHBG concentrations ($r = .381$, $p = .000$; $r = .215$, $p = .033$; $r = .286$, $p = .004$, respectively), while a negative relationship was observed between LH and InhB concentration ($r = -0.404$, $p = .000$). FSH concentrations were negatively associated with fT and InhB concentrations ($r = -0.287$, $p = .004$; $r = -0.566$, $p = .000$, respectively), while T concentrations were positively associated with fT, E_2, and SHBG concentrations ($r = .506$, $p = .000$; $r = .246$, $p = .015$; $r = .493$, $p = .000$, respectively).

Further, T concentration was negatively correlated with TG concentration ($r = -0.313$, $p = .002$). A positive correlation was found between serum E_2 and HDL-C concentration ($r = .282$, $p = .005$), and a negative correlation was found between serum E_2 and LDL-C ($r = -0.229$, $p = .024$). A significantly negative correlation was found between serum SHBG and TG concentrations ($r = -0.276$, $p = .006$). The data showed a significantly positive correlation between serum TC and LDL-C concentration ($r = .871$, $p = .000$), and a significantly negative correlation between serum HDL-C and LDL-C concentration ($r = -0.245$, $p = .015$). More information on the results of the correlation analysis of age, BMI, and serum hormonal and lipid concentrations is provided in the Supplementary data.

Correlations of Semen Quality Variables With Age, Anthropometric, Hormonal, and Lipid Parameters

There were no significant correlations between age and semen volume, concentration, total sperm count, progressive motility, and normal morphology. No significant relationships were seen between obesity-associated markers (waist and hip circumference, WHR, WHtR, and BMI) and semen parameters.

Significant negative relations were revealed between LH level and sperm concentration, and progressive sperm motility ($r = -0.273$, $p = .008$; $r = -0.265$, $p = .013$, respectively); significant positive relations were revealed between InhB level and sperm concentration, and progressive sperm motility ($r = .290$, $p = .006$; $r = .346$, $p = .001$, respectively). There were no significant relationships between lipid and sperm parameters. More information on the results of the correlation analysis of semen quality variables, age, anthropometric, hormonal, and lipid parameters is provided in the Supplementary data.

Distribution of Hormonal and Lipid Concentrations According to Semen Quality

The next step of our study was to compare the hormonal and lipid levels based on the different semen quality. Participants were divided into two groups: one with normal and the second with impaired semen parameters in accordance with the WHO reference limits (World Health Organization, 2010) for sperm concentration (<15.0 million/ml), progressive motility (<32%), and morphology (<4.0%). Semen and hormonal and lipid variables were adjusted for the period of sexual abstinence and age. Results are shown in Table 3.

The LH concentration was higher, but the InhB concentration was lower in the group with impaired sperm parameters than in the group with normal semen quality. The results showed that the concentrations of other reproductive hormones, SHBG, and lipids did not differ between the two groups. We failed to show differences between the two groups on obesity-associated markers (waist and hip circumference, WHR and WHtR, and BMI; the data were not presented).

Discussion

Geographical variability in semen quality may be biologically meaningful, affecting fertility and reproductive health of the population. Significant geographical differences in

Table 3. Comparisons of Hormonal and Lipid Concentrations Based on Different Semen Quality.

	Group with normal semen quality ($n = 55$)		Group with impaired semen quality ($n = 35$)	
Variable	Mean (*SD*)	Median (5–95)	Mean (*SD*)	Median (5–95)
Semen volume, ml	3.3 (1.8)	2.9 (1.1, 5.7)	3.2 (1.7)	3.1 (1.1, 1.8)
Sperm concentration, $\times 10^6$/ml	75.74 (47.84)	53.25 (25.37, 164.98)	24.49[a] (13.99)	22.80 (0.63, 52.53)
Total sperm count, $\times 10^6$	232.1 (161.9)	175.65 (51.4, 585.4)	86.5[a] (79.5)	57.2 (2.0, 246.8)
Progressive motility, %	60.6 (19.1)	57.7 (33.6, 95.5)	19.4[a] (13.9)	18.5 (2.0, 53.0)
Normal morphology, %	8.38 (2.50)	8.25 (5.00, 13.75)	4.56[a] (2.06)	4.25 (1.75, 8.25)
LH, mIU/ml	**3.14 (1.34)**	**2.90 (1.44, 5.47)**	**3.83[b] (1.37)**	**3.82 (2.14, 7.11)**
FSH, mIU/ml	3.79 (3.06)	3.17 (1.29, 9.02)	4.55 (2.60)	4.25 (1.03, 10.24)
Total testosterone, nmol/L	17.08 (7.25)	15.15 (7.67, 30.91)	17.08 (8.16)	14.22 (8.58, 36.30)
Free testosterone, pg/ml	12.36 (5.97)	12.20 (3.70, 21.30)	13.24 (10.97)	9.70 (3.90, 28.70)
Estradiol, nmol/L	0.173 (0.036)	0.166 (0.129, 0.240)	0.184 (0.047)	0.180 (0.116, 0.297)
Inhibin B, pg/ml	**214.4 (42.9)**	**207.3 (151.7, 294.4)**	**186.4[b] (52.6)**	**186.9 (100.0, 300.3)**
SHBG, nmol/L	51.55 (27.55)	43.60 (21.60, 126.90)	52.66 (38.86)	38.30 (19.50, 172.50)
TG, mmol/L	1.31 (0.64)	1.08 (0.39, 2.54)	1.40 (0.81)	1.15 (0.41, 2.89)
TC, mmol/L	4.40 (0.88)	4.25 (3.25, 6.15)	4.55 (1.24)	4.39 (2.50, 6.71)
HDL-C, mmol/L	1.14 (0.36)	1.06 (0.65, 1.78)	1.21 (0.42)	1.06 (0.66, 2.04)
LDL-C, mmol/L	2.69 (0.92)	2.58 (1.51, 4.89)	2.70 (1.15)	2.73 (0.86, 5.14)

Note. Results are based on raw data. SD = standard deviation; (5–95): 5th–95th percentile; LH = luteinizing hormone; FSH = follicle-stimulating hormone; SHBG = sex hormone-binding globulin; TG = triglyceride; TC = total cholesterol; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.
[a]$p \leq .001$. [b]$p \leq 0.05$. *P* values were obtained from analysis of covariance (ANCOVA) taking confounders into consideration.

sperm parameters are observed not only between different countries, but also between different regions of the same country, indicating a multifactor reason for these differences. In the Nordic-Baltic area, Norwegian and Danish men from the general populations had lower sperm concentrations and percentage of morphologically normal sperm compared with Estonian or Finnish men, indicating an east-west gradient in sperm quality (Jørgensen et al., 2002). Genetic and/or environmental factors have been proposed as the possible causes of this gradient. The differences for morphologically normal and motile spermatozoa were observed between young men from the general population of Hamburg and Leipzig in Germany (Paasch et al., 2008). The authors suggested that a heavily polluted environment in the region of Leipzig might play a major role. The population-based study performed in the United States showed that sperm concentration and total number of motile sperm were lower in males from Columbia compared with citizens from New York, Minneapolis, or Los Angeles (Swan et al., 2003). The authors believed that sperm concentration and motility might be reduced in semirural and agricultural areas relative to more urban and less agriculturally exposed areas. The regional differences in sperm concentration and motility in military personnel were revealed between six different geographical areas of China suggesting that diet, lifestyle, climate, and altitude could be possible contributory factors (Zou et al., 2011). The population study of university students showed no differences in semen quality between men from four different provinces in Japan, in that the semen results for Japan men were better than reported for young men from some Northern European countries, indicating racial peculiarities (Iwamoto et al., 2013).

To our knowledge, our study is the first that to investigate the semen quality of Russian men from the general urban population residing in the European North of Russia. It was interesting to compare the semen results of Russian men with those of men from other neighboring Northern or Eastern European countries geographically close to the European North of Russia, taking into consideration that results could be obtained by different methods of semen analysis. International comparisons of semen quality may be also limited by other factors, including age, various criteria for inclusion of participants into the study population, ethnicity and genetic backgrounds, environmental conditions, and others.

Semen parameters published for men from the general population of nine neighboring Northern or Eastern European countries are shown in Table 4. The median semen volume did not differ between our and other studies from Northern or Eastern Europe. In all papers listed in Table 4, the semen volume was estimated by weighing the collection tube and subsequently subtracting the predetermined weight of the empty tube assuming 1 g = 1 ml. Exception was one paper, where the semen volume from Lithuanian men was measured in a

Table 4. Summary of Semen Parameters in Men From the General Population Living in Northern or Eastern European Countries.

Reference	Region	Age, years	Semen parameters, Mean (Median)			
			Semen volume, ml	Sperm concentration, $\times 10^6$/ml	Sperm motility, %	Morphologically normal sperm, %
Jørgensen et al., 2002	Finland (n = 324)	military conscripts 18	3.3 (3.0)	72 (61)	64 (66)[a]	8.9 (9.0)[c]
	Estonia (n = 104)		3.2 (3.1)	72 (62)	73 (75)[a]	9.2 (9.0)[c]
	Norway (n = 240)		3.1 (2.9)	69 (53)	64 (66)[a]	6.9 (7.0)[c]
	Denmark (n = 300)		3.3 (3.0)	57 (44)	65 (68)[a]	6.4 (5.0)[c]
Punab et al., 2002	Lithuania (n = 196)	18	2.8 (2.5)	75 (65)	76 (79)[a]	6.2 (6.0)[c]
	Estonia (n = 79)	19	3.3 (3.0)	81 (64)	74 (76)[a]	7.7 (7.0)[c]
Malm et al., 2004	Norway:	19–40	– (3.6)	– (53)	– (48)[b]	–
	Oslo (n = 112)		– (3.7)	– (62)	– (50)[b]	
	Tromsoe (n = 92)					
Paasch et al., 2008	Germany:	military conscripts 19.7 18.9	3.4 (3.2)	63 (49)	66 (68)[a]	9.3 (8.5)[c]
	Hamburg (n = 334)		2.7 (2.6)	65 (45)	77 (82)[a]	8.3 (8.0)[c]
	Leipzig (n = 457)					
Axelsson et al., 2011	Sweden (Malmo area) (n = 295)	17–20	2.9 (2.7)	71 (56)	53 (58)[b]	–
Jørgensen et al., 2012	Denmark (Copenhagen area) (n = 4867)	18–19	3.4 (3.2)	60 (45)	65 (68)[a] 56 (59)[b]	7.1 (6.5)[c]
Halling et al., 2013	Faroe Islands (n = 481)	24	4.1 (3.9)	57 (40)	64 (64)[a]	6.9 (6.5)[c]
	Denmark (n = 1274)	19	3.6 (3.3)	62 (48)	65 (68)[a]	7.5 (7.0)[c]
Kamieniczna et al., 2015	Poland:	18–35	3.5 (–)	49.6 (–)	42.3 (–)[b]	–
	Poznan (n = 113)		3.1 (–)	40.9 (–)	47.5 (–)[b]	
	Lublin (n = 89)					
Erenpreiss et al., 2017	Estonia:	16–29	– (3.0)	– (68)	– (57)[b]	– (11.5)[c]
	Russians (n = 272)		– (3.4)	– (63)	– (59)[b]	– (12.0)[c]
	Estonians (n = 301)		– (3.2)	– (55)	– (61)[b]	– (11.5)[c]
	Latvia (n = 278)		– (3.4)	– (63)	– (60)[b]	– (12.0)[c]
	Lithuania (n = 314)					
Our study	***European North of Russia (n = 90)***	**21–63**	***3.3 (3.0)***	***55.8 (42.1)***	***45.0 (43.8)***[b]	***7.0 (6.5)***

Note. [a]The sum of progressively motile (classes A + B) and local motile (class C) sperm. [b]WHO progressive motility (classes A + B). [c]Semen morphology was defined according to "strict criteria" (Menkveld et al., 1990).

graded tube (Punab et al., 2002). In addition, the median semen volume of Russian men from the European North of Russia coincided with that of Russians from Estonia (Erenpreiss et al., 2017).

The sperm concentration of Russian men seemed to be lower when compared to the men from the general urban populations from other Northern or Eastern European countries (Table 4). Sperm concentration was assessed using the hemocytometer in all papers listed in Table 4; it allows for a comparison of the results from these regions. The median sperm concentration reported in our study was obviously lower than that in the men from Finland, Estonia, Lithuania, Norway, and Sweden (Axelsson et al., 2011; Jørgensen et al., 2002; Malm et al., 2004; Punab et al., 2002). Russians from the European North of Russia seemed to have lower sperm concentration compared to Russians from Estonia (Erenpreiss et al., 2017). The median sperm concentration was similar to that of residents from Denmark, Germany, or Faroe Islands (Halling et al., 2013; Jørgensen et al., 2002, 2012; Paasch et al., 2008).

Semen quality and quantity have been reported to decline with increasing age (Eisenberg & Meldrum, 2017; Gunes et al., 2016). Age of participants was different in Russian and European men from most Northern or Eastern countries. The participants in our study were older than the participants of other European studies. The relationship between age and sperm parameters was previously studied in the same group of Russian men from the European North of Russia, but the age effect (in range 21–63 years) was not found (Osadchuk et al., 2019). In addition, there were no correlations between age and semen volume, concentration, total sperm count, progressive motility, or normal morphology in the current study. Thus, our results suggest that

participants' age was not a key factor determining regional differences in semen quality between Russian and other European populations.

In the papers listed in Table 4, sperm motility was assessed as progressive motility (classes A + B) according to the WHO laboratory manual (World Health Organization, 2010) or as the sum of progressive and local motile sperm (classes A + B + C). The automatic sperm analyzer was used to assess sperm progressive motility (classes A + B) in our study, which makes it difficult to compare the results. Despite the different methods of sperm motility analysis, the sperm progressive motility of the men from Russia was very close to the values reported for Norwegians or Poles (Kamieniczna et al., 2015; Malm et al., 2004), but the sperm progressive motility in Russians was lower than in men from Sweden, Denmark, Estonia, Latvia, and Lithuania (Axelsson et al., 2011; Erenpreiss et al., 2017; Jørgensen et al., 2012). Progressive motility differed between Russians from the European North of Russia and Estonia; it was better in Russians from Estonia (Erenpreiss et al., 2017).

In all the papers listed in Table 4, the sperm smears for morphology evaluation were stained by Papanicolaou and assessed according to "strict criteria" (Menkveld et al., 1990), but we used Diff-Quick for staining the slides and examined for sperm morphology according to the WHO laboratory manual (World Health Organization, 2010). The Diff-Quick staining has been chosen as the fastest method for staining ejaculate smears; this method is already widely used in clinical andrology and epidemiological studies to assess sperm morphology with standard bright field microscopy. Our observations and other studies showed that quality of staining was satisfactory for a rapid assessment of normal forms in most samples (Crazzolara et al., 2007; Kruger et al., 1987; Meeker et al., 2007). In addition, no significant differences were observed for the percentage of morphologically normal sperm when comparing the Papanicolaou and Diff-Quick staining methods (Kruger et al., 1987). A recent study demonstrated that Diff-Quik stain could also serve easily and routinely to give information on the status of chromatin in human sperm (Sousa et al., 2009).

The WHO main recommendations for the assessment of human sperm morphology is based on the Tygerberg Strict Criteria (Björndahl et al., 2016), which makes it possible to compare the results from our study with those of other studies shown in Table 4.

The median percentage of sperm with normal morphology in residents from the North of Russia was lower than in residents from Finland, Estonia, Latvia, Norway, and Germany, but was very close to Danish, Lithuanian, and Faroese men and also to Russians from Estonia (Halling et al., 2013; Jørgensen et al., 2002, 2012; Punab et al., 2002). Thus, regional comparisons showed an impaired semen quality in Russian men including sperm concentration, motility, and normal morphology than in the men from most neighboring European countries. Although a possible explanation for regional differences may be related to environmental conditions, diet, or lifestyle rather than age or methodological differences, more detailed investigations are needed to explore the potential impact of these factors.

According to our data, approximately 40% of Russian men had at least one of the semen parameters (sperm concentration, motility, or normal morphology) below the WHO reference limits (World Health Organization, 2010). The data obtained raise a concern of lower sperm quality of residents from the European North of Russia. Similarly, a large proportion of men from the general population with abnormal sperm parameters was revealed in Denmark and China (Jørgensen et al., 2012; Zou et al., 2011). In a large one-center prospective study of men from the general Danish population, it was found that only one in four men had optimal semen quality, approximately 25% had a reduced quality and 15% had severely impaired quality (Jørgensen et al., 2012). In a large sample of military personnel aged between 18 and 35 years from different geographical areas of the People's Republic of China, it was found that 62.5% of men had at least one semen parameter below normal values according to WHO reference limits (Zou et al., 2011). It should be noted that the WHO reference limits for sperm parameters are not minimal threshold values for conceiving, and fertile men can have semen parameters lower than the WHO reference limits (Cooper et al., 2010).

Spermatogenesis requires the action of a complex set of peptide and steroid hormones, but they cannot serve as valid substitutes for the semen parameters used in epidemiological population studies. Lowered serum concentrations of total testosterone and higher LH can be revealed in a subpopulation of infertile men compared with levels in fertile men (Andersson et al., 2004b). However, the total testosterone levels were not related to the sperm parameters in young European men from the general population; the reduced sperm quality was accompanied by an increased level of LH indicating a compensated reduction in Leydig cell function (Jørgensen et al., 2016). Despite the fact that FSH is considered the main endocrine marker of spermatogenesis, some authors doubt the predictive ability of FSH due to its substantial variability and insufficient diagnostic accuracy (Andersson et al., 2004a). Inhibin B regulating FSH secretion via a negative feedback is an additional diagnostic parameter as a direct marker of Sertoli cells function and as an indirect one of spermatogenesis (Barbotin et al., 2015; Iliadou et al., 2015). A positive relationship between the inhibin B level and the concentration/total number of sperm in the ejaculate is considered to be

proven (Barbotin et al., 2015); however, a significant overlap of FSH and inhibin B levels is observed between infertile men and men from the general population (Andersson et al., 2004a).

The median FSH and inhibin B levels in our study were similar to those of men from neighboring Baltic countries (Jørgensen et al., 2002; Punab et al., 2002). However, the LH and total testosterone level were lower in Russian men compared to men from Baltic countries (Jørgensen et al., 2002; Punab et al., 2002). The same situation was observed when comparing Russian men and the residents of the Faroe Islands (Halling et al., 2013): the FSH and inhibin B levels were similar, but the LH and total testosterone levels were lower in the former than in the latter case. The regional comparisons of LH and total testosterone levels indicated a bit weaker function of Leydig cell function in the men from the European North of Russia compared to the men of the Nordic-Baltic area. In addition, the negative relationship between LH level and sperm concentration, and progressive motility, as well as the positive relationship between inhibin B level and sperm concentration, and progressive motility were obtained in our study. Similar data were obtained by other European studies (Andersson et al., 2004a, 2004b; Kamieniczna et al., 2015). Jørgensen et al. (2016) reported that in the Danish general population, the negative correlation of LH and sperm concentration might have been a result of impaired Leydig cell function and compensative increase of LH production by the hypophysis. Thus, the conducted comparisons suggest that lower sperm quality in the Russian men might be associated with impaired Leydig cell function. One of the possible reasons of decreased levels of LH and testosterone in Russian men compared to the results of other European studies may be a larger proportion of overweight and obese participants. Actually, a significant portion of our study group (63.6%) had increased BMI, so the median BMI (26.5) was higher than reported for participants of the Nordic-Baltic or German studies (Erenpreiss et al., 2017; Halling et al., 2013; Jørgensen et al., 2012; Paasch et al., 2008). In a previous study on the same group of participants, we were not able to demonstrate the effect of BMI on semen parameters (Gutorova et al., 2014). The present study did not reveal any significant correlations between BMI or other obesity-associated anthropometric markers and semen parameters. However, our results showed that BMI was negatively related to serum levels of total testosterone, estradiol, and SHBG that were unrelated to semen parameters; on the other hand, serum levels of LH and inhibin B significantly affected semen parameters, but had no correlations with BMI. These facts may explain why BMI might affect serum levels of some reproductive hormones, but did not change semen parameters.

The data regarding relationships between semen parameters and reproductive hormone levels in men from the general population are very scarce. To investigate a relation between the Leydig cell function and semen quality, Jørgensen et al. (2016) examined young men (median age 19 years) from the general populations in seven European countries ($n = 8{,}182$). They found that serum testosterone levels were not associated with sperm concentrations, total sperm counts, or percentage of motile or morphologically normal sperm. They showed that there were inverse associations between semen parameters and serum LH levels. In another report, a shift toward lower serum testosterone levels and higher serum LH levels was observed in the group of infertile men ($n = 357$), indicating significant signs of impaired Leydig cell function in parallel to their impaired spermatogenesis (Andersson et al., 2004b). The authors suggested that in men with impaired spermatogenesis, normal testosterone levels might sustain in the background of slightly elevated LH levels assuming a compensated dysfunction of Leydig cells in these men. We also found a significant negative correlation between serum LH levels and sperm concentration, and progressive motility. Additionally, a higher serum LH level was obtained in the group with impaired semen quality compared to the group with normal semen quality. These data are in agreement with data published by Andersson et al. (2004b) and Jørgensen et al. (2016), and suggest that the impaired sperm quality in the men of the European North of Russia might be associated with the impaired Leydig cell function.

In our study, we evaluated the association between men's serum lipid concentrations and semen quality parameters. Despite the fact that 45.5% of participants of the study group were characterized by dyslipidemia, we were not able to establish reliable relationships between semen parameters and serum lipids levels, as well as differences in the lipid profile between groups with normal and impaired sperm parameters. A small number of studies have focused on the associations between serum lipid profiles and semen quality, but conclusions and results reported were inconsistent. One prospective cohort study reported that hypercholesterolemia was not associated with semen quality in a cohort of 456 men, with a mean age of 31.8 years (Eisenberg et al., 2015). Another study reported there was no correlation between sperm concentration and serum total cholesterol or triglyceride in 631 Chinese subfertile men (Lu et al., 2016). The results of these studies were consistent with the conclusions of our study suggesting that serum lipids levels might not reflect sperm quality including sperm concentration, motility, and normal morphology.

Correlation of seminal parameters with serum lipid profile have been studied in 18 infertile men (Ergün et al., 2007). In this study, the increased serum very

low-density lipoprotein and total triglyceride values were significantly correlated with decreased sperm motility. In a later clinical study, higher levels of serum total cholesterol, free cholesterol, and phospholipids were associated with a significantly lower percentage of sperm with normal head morphology among 501 male partners of couples desiring pregnancy (Schisterman et al., 2014). In 167 Japanese male partners of infertile couples aged 22–46 years, there was no significant relationship between serum total triglyceride level and sperm concentration or motility; however, the serum total triglyceride level was positively associated with the sperm morphological traits (Hagiuda et al., 2014). The associations between different lipid profiles and semen quality were explored in a large-scale general male population of 7601 participants (Liu et al., 2017). Sperm concentration was positively correlated with triglyceride and very low-density lipoprotein, and progressive motility was statistically increased with increasing low-density lipoprotein and cholesterol levels. These results seemingly conflict with findings of our and other reports, which did not find the evidence for association between serum lipid concentrations and semen quality.

The reasons for the various relationships between serum lipids and sperm quality remain unclear. Lu et al. (2016) concluded that the potential effects of lipids on semen quality could be better observed when seminal lipid levels were used as a marker compared with serum lipid levels. Seminal plasma triglyceride, total cholesterol, low-density lipoprotein, and high-density lipoprotein levels in patients with impaired spermatogenesis were higher than in patients with normal sperm concentration, motility, or morphology. An investigation of the lipid composition of human semen showed a significant higher cholesterol sulfate/seminolipid ratio in semen of oligoasthenozoospermic patients than in subjects with normal motility values (Lopalco et al., 2019). In line with the above reported data, another study showed that the cholesterol level in seminal plasma was positively associated with sperm concentration, total sperm count, sperm motility, and morphology, but there was no association between serum cholesterol and the tested semen parameters (de Neergaard et al., 2018). The positive associations in that study were observed in men with normal serum cholesterol levels, and there was no association between serum and seminal plasma levels of cholesterol. Further study is needed to clarify the exact role of seminal plasma lipid levels for semen quality to corroborate or refute a suggestion about links between an increased prevalence of dyslipidemia and poorer semen quality in males from the European North of Russia.

There are a few limitations to our study. First, the sample size was relatively small and could result in attenuated statistical power, wider confidence intervals, or risks of errors in statistical analysis. Relatively low study participation rate determined the small number of men in our study group. It has to be stressed that the recruitment of participants proved to be very difficult. Since all participants were volunteers, this could cause a selection bias and skew the results of our study keeping in mind the "healthy worker" effect that might have taken place; however, a similar selection bias could have taken place in many other investigations as well. If the majority of epidemiological studies on sperm quality included participants with proven fertility or from infertile couples, then our sample of study subjects included men with unknown fertility who did not have information about their sperm parameters. Thus, the "random sampling" approach we used can be considered as representative of the general population. In addition, studies of sperm quality based on random samples from the general population are very few and of great interest in terms of ethnic and regional differences.

Another limitation of the study was the inability to adjust for some possible confounders, including some lifestyle factors that would have been beneficial for interpretation of relationships between sperm quality, and hormonal and metabolic parameters; hence, the findings need to be interpreted with caution. Some studies suggest that cigarette smoking and alcohol intake is associated with reduced semen quality, but moderate physical activity is favorable for improvement of semen quality (Durairajanayagam, 2018; Hayden et al., 2018). We failed to use questionnaire information on the lifestyle factors because it may inadequately reflect actual data.

The strength of our study was that our participants were a homogeneous group relative to the environment in which they lived. Most of them were born and living in the Arkhangelsk region; and have been exposed to the same environmental and cultural factors existing in a circumpolar area; therefore, in this sense, our study group exactly reflected the general population. All the samples were obtained during a single winter month, which avoids any seasonal influences. In addition, the sampling time was standardized; all samples were drawn in the morning and were processed by the same scientific team using standardized laboratory techniques. Our semen and hormonal results may serve as the point of departure for further studies of Russian men from the general population, taking into account the wide diversity of climatic conditions and nationalities in Russia.

In conclusion, our results are the first to describe the semen quality of Russian men residing in the circumpolar area, which can be very useful for future studies on the reproductive potential of other Russian populations. Comparisons of semen and hormonal results with those published for the neighboring Northern or Eastern European countries confirmed the existence of regional

differences even between regions with close climatic conditions. The sperm quality in Russian men was slightly worse than in men from Finland, Norway, Sweden, or Estonia, but very similar to that in men from Denmark or Poland. Our results showed that a significant proportion of Russian men from the general population had suboptimal semen quality, suggesting reduced fertility potential. Despite the fact that more than 45.5% of participants had dyslipidemia, serum lipid levels were not associated with semen quality. We have shown that the semen quality parameters were inversely associated with serum LH but not testosterone levels in the men from the general population. These data suggested that a lower sperm quality in Russian men might be associated with an impairment of the Leydig cell function due to a larger proportion of overweight and obese participants.

Authors' Note

All study subjects were volunteers and did not received any financial compensation. The Ethical Committee at the N. Laverov Federal Center for Integrated Arctic Research, Arkhangelsk, approved the study.

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Author Contributions

LO: Provided overall supervision of the project, hormonal and metabolite analysis; interpreted the data; and wrote the manuscript. ET: Organized the experiments; performed free testosterone and SHBG analysis. IG: Collected questionnaires and samples; carried out the experiment. AO and MK: Performed the semen and statistical analysis; participated in drafting the manuscript.

Declaration of Conflicting Interests

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Supplemental Material

Supplemental material for this article is available online.

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