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ORIGINAL ARTICLE

Comparison of selected sperm parameters between 6,278 males in Poland and Ukraine

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Abstract

The phenomenon of deterioration of the quality of sperm in Poland and Ukraine has not been unequivocally evidenced by scientific research. Spermiogramms of males reporting for the first time to infertility treatment centres in both countries were examined, and trends in changes of selected sperm parameters analyzed during the period of study. Analyses covered the spermiograms of 6,278 men living in the examined regions for at least 3 years. It was found that mean sperm concentration of patients in the Lublin and the Lvov regions did not differ, but the total mean number of spermatozoa produced by Poles was higher than in Ukrainians. The quality of semen with respect to motility and sperm morphology was better in Ukrainian than Polish patients. Despite differences concerning concentration, the amount of sperm produced and percentage of spermatozoa showing progressive motility which occurred between individual years, no clear tendency was observed towards change of these parameters in a specified direction in both countries during the years examined. Among men examined in the Lublin Region, a tendency was noted towards decrease in the percentage of morphologically normal spermatozoa in the ejaculate, while among patients from the Lvov Region an opposite tendency was observed.

Key words

Poland, Ukraine, semen quality, decline

INTRODUCTION

The problem of the lack of offspring affects nearly every fifth couple at reproductive age in Europe, and in many cases is caused by the male factor [1]. Many environmental factors and life style exert an effect on male fertility [2, 3]. The presence and activity in the life and work environment of hazardous factors which impair the male reproductive function may lead to disorders in the process of spermatogenesis, and impair the production of good quality reproductive cells [4]. These factors, to a great extent, depend on the place where a man normally lives, and are shaped by the development of civilisation. Many scientific reports suggest that over the last several dozen years male fertility has been systematically decreasing, although the causes of the occurrence of this phenomenon remain unknown [5, 6].

Semen analysis are, among other things, the reflection of male reproductive capabilities, and constitute a basic criterion for the evaluation of male fertility. Within the last dozens of years, together with a decrease in the parameters of male sperm, the lower limits of normal sperm concentration and motility in ejaculate have decreased, assessed according to the WHO 2010 criteria [7]. Further intensification of this process may contribute to a rapid growth in the number of couples at reproductive age who will struggle with the problem of infertility in the near future [8].

The phenomenon of deterioration of the quality of sperm in Poland and Ukraine has not been unequivocally evidenced by scientific research; however, alarming trends in this respect occurring in Europe may provide basis for expectations that this problem will also concern these two countries [9, 10, 11]. Recently, Poland and Ukraine experienced many tumultuous political and civilization changes. The study concerned two adjacent regions in these countries, possessing similar geographic conditions. From Medieval times, for about 500 years, these territories co-existed within one country, and at the end of the 18th century, as a result of military actions and partitions of Poland, were divided by a border. From that time (except for the 20-year period between the First and Second World Wars), the communities living in these areas have developed in different conditions. In 2004, Poland joined the European Union, which resulted in a quicker economic development, but also changes in the environment of life and work [12]. The living standard of Poles has improved and become closer to that in the rest of Europe [13]. After Poland entered the Schengen area on 21 December 2007, the migration of citizens between Ukraine and Poland has been considerably limited. Polish society develops in conditions shaped by the European Union, while Ukraine follows another path, and while writing this paper remains in the state of war with so-called separatists. The existing military conflict interrupted the presented study.

The aim of the study was to compare the spermiogramms of males reporting for the first time to infertility treatment centres in Lublin (Poland) and in Lvov (Ukraine). Over the analyzed period, trends in changes of selected sperm parameters were also examined.

MATERIAL AND METHODS

The study was a retrospective analysis performed in 2014, based on the materials obtained from the Clinic of Reproduction and Andrology in Lublin (Poland), and the Intersono Medical Centre in Lvov (Ukraine). Analyses

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covered the spermiogramms of men who reported for the first time to these centers for infertility treatment. Sperm analysis was assessed manually under a microscope during the period 2005–2009, in accordance to the WHO 1999 criteria, and later according to the WHO criteria of 2010 after 3–6 days of sexual and alcohol abstinence (World Health Organization, 1999; World Health Organization, 2010) [14, 15]. We assumed that "forward progression" mean the same as "grade A+B sperm motility" in WHO (1999) and "progressive motility" in WHO (2010). In the Polish site, sperm examination was carried out by the same people with "lab analyst" education, who were externally audited by Nordic Association for Andrology, whereas internal audit was performed every two months (two people were cross-checking themselves). Polish analysts audited Ukrainian analysts every six months (two people during the whole examination), and the latter submitted themselves to internal audit every two months as far as proper sperm examination is considered. The men were aged 20–60, and were all permanent residents of the Lublin or Lvov regions living in these regions for at least 3 years – 3,266 in Poland, and 3,012 in Ukraine (Fig. 1). Men with genetic and systemic diseases were excluded from the study. The studies were approved by the Ethics Committee.

The results of the study obtained were subjected to statistical analysis. The values of the analyzed parameters were presented using the mean value. The differences between Poland and Ukraine were investigated using Mann-Whitney U test and t-Student test, while in order to test differences in the variables analyzed between the subsequent years, the analysis of variance ANOVA and the NIR test were applied. The p values of $p<0.05$ were considered statisticaly significant. The database and statistical analysis were performed based on the software Statistica 9.1 (StatSoft, Poland).

RESULTS

Mean age of patients from Poland was 32.28, while in those from Ukraine 32.93 ($t=-4.53$, $p<0.001$). Although the difference was statistically significant, it seems not to have influenced the obtained examination results from the clinical standpoint.

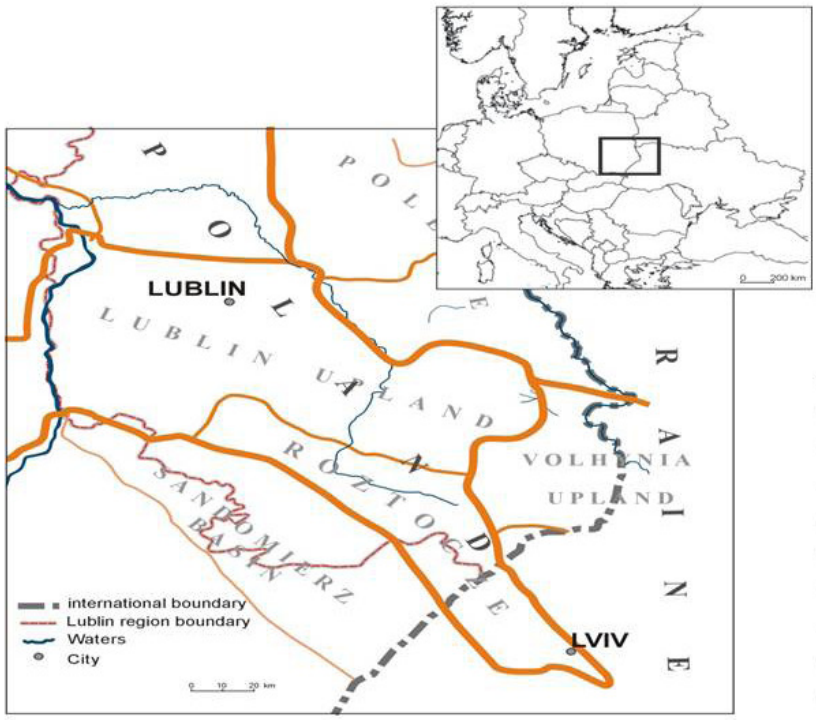
Figure 2 demonstrates the comparison of mean sperm concentration, total number of spermatozoa, percentage of spermatozoa showing progressive motility and morphologically normal spermatozoa in the examined ejaculates between Polish and Ukrainian men.

While comparing the sperm concentration of 3,266 patients from Poland, and 3,012 from Ukraine, no statistically significant difference was found ($t=-0.34$; $p=0.734$) between the mean number of spermatozoa per 1 millilitre. The mean sperm concentration in Poland was 36.33×10^6/ml, whereas in Ukraine this value was 36.02×10^6/ml.

The mean total number of spermatozoa in semen was also compared in these patients, and statistically significant differences were found between the groups examined ($t=5.87$, $p<0.001$). In Poland, the mean total number of spermatozoa in the ejaculate was 145.69×10^6, and was higher than in males from Ukraine – 123.75×10^6.

While comparing 3,117 samples of ejaculates containing spermatozoa from Poland with 2,913 samples from Ukraine statistically significant differences were found in the mean percentage of spermatozoa showing progressive motility. The value of this parameter was higher in the sperm of men from Ukraine, and was 27.43%, while in Poland – 19.48 % ($t=-18.19$ $df=6028$, $p<0.001$).

Also, the mean percentage of morphologically normal spermatozoa was also compared, evaluated according to the criteria by the WHO 2010, among 1,566 patients from Poland, and 2,225 from Ukraine. Statistically significant differences



Year	Ukraine [N]	Poland [N]
2005	0	301
2006	0	238
2007	0	239
2008	0	445
2009	786	409
2010	530	482
2011	750	515
2012	631	459
2013	315	180

Figure 1. Number of patients in individual years and place of study

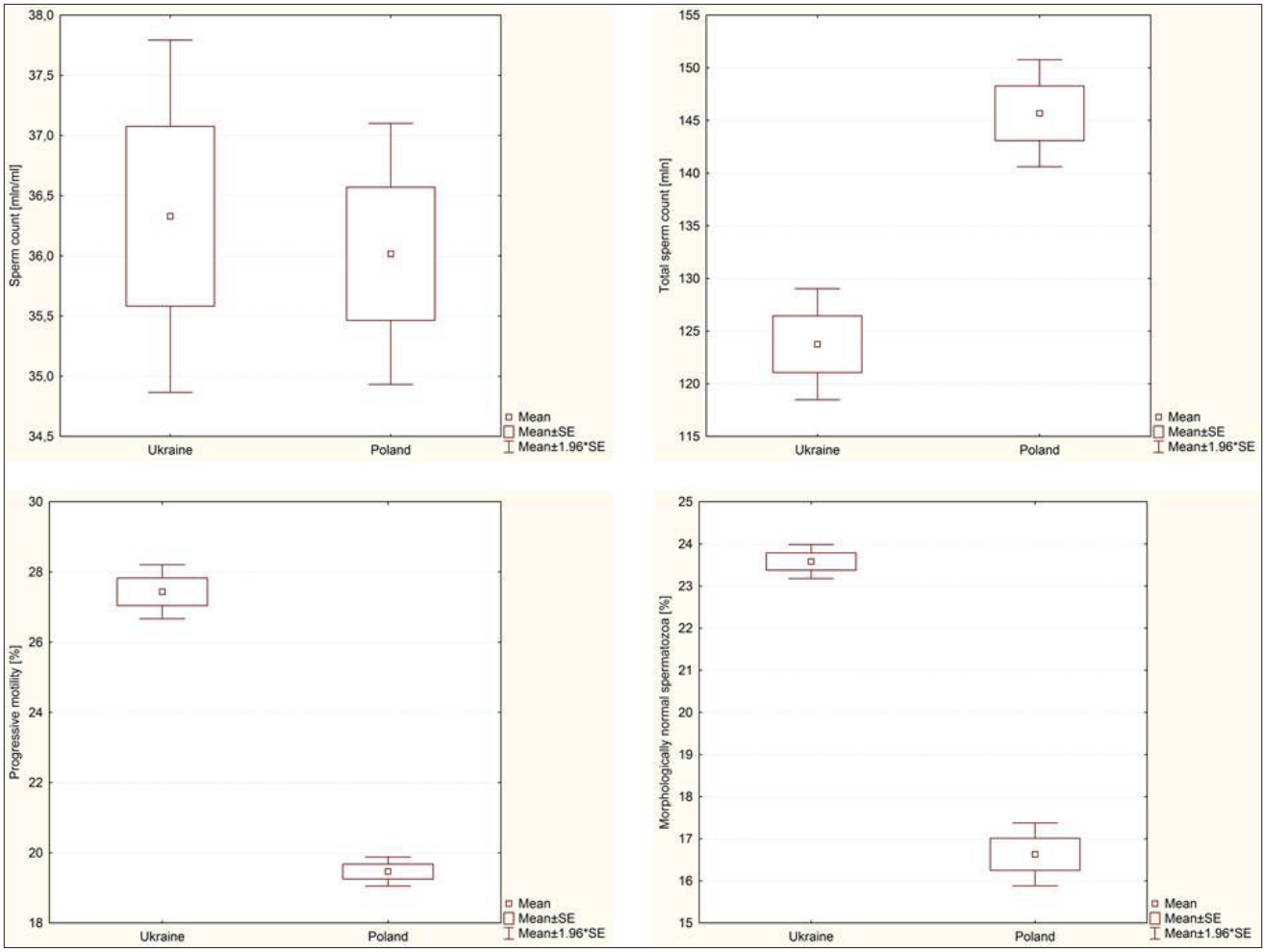


Figure 2. Comparison of mean sperm concentration, total number of spermatozoa, percentage of spermatozoa showing progressive motility and abnormal sperm in the examined ejaculates between Polish and Ukrainian men. Abbreviations: SE – standard error

were found between these two groups (Z=18.22, p<0.001). Among Polish males, the percentage of morphologically normal spermatozoa remained on the mean level of 16.65%, whereas among Ukrainian men it was 24.36%.

Between the years 2005–2010, in Polish patients, the mean sperm concentration and the total number of spermatozoa showed an upward tendency, and subsequently started to decrease gradually. Analysis of variance of these parameters indicated the presence of statistically significant differences between years (concentration F=25.31; p<0.001, total number F=24.79; p<0.001). Considering the mean sperm concentration, statistically significant differences concerned the years 2005/2006, 2007/2008, 2008/2009, 2010/2011, and 2011/2012. Similar relationships concerned the total number of spermatozoa; however, in this case, statistically significant differences additionally concerned the years 2009/2010, while for the remaining years no statistically significant differences were observed. Among Polish men, the mean percentage of spermatozoa with progressive motility did not show any clear tendencies towards changes in a specified direction, and assumed values from 17.61%–21.86%. Analysis of variance of this parameter indicated the presence of significant differences between the years examined (F=4.75, p<0.001). With respect to the percentage of progressive motility statistically significant differences were noted between the years 2007/2008, 2008/2009, and 2011/2012. In the case of mean percentage of morphologically normal spermatozoa in Poles according to the WHO 1999 criteria, a gradual decrease in this parameter was observed from 2005/2009, with a temporary increase in 2008. Analysis of variance showed the presence of statistically significant differences between the years examined (F=33.37; p<0.001), based on the NIR test, it was found that these differences concerned subsequent years. In Polish patients, the mean percentage of morphologically normal spermatozoa determined according to the WHO 2010 criteria decreased within the period 2010/2013. Analysis of variance of this parameter showed the occurrence of statistically significant differences between the years (F=87.698; p<0.001), and the NIR test indicated that statistically significant differences occurred from 2010/2012, whereas no statistically differences were noted between 2012/2013 (Tab. 1).

Among men from Ukraine, the mean sperm concentration and number of spermatozoa in ejaculates reached the highest value in 2009, while the lowest in 2012, ranging between these years. Based on analysis of variance it was confirmed that the differences in these parameters occurring between years are statistically significant (concentration F=133.97; p<0.001; total number F=123.96; p<0.001), and based on the NIR test it was found that these differences concerned subsequent years. Among Ukrainian men the mean percentage of spermatozoa with progressive motility changes significantly

Table 1. Changes in sperm parameters during the examined years among Poles

Sperm concentration [x10^6/ml]										Total sperm concentration [mln]									
Analysis of variance		Significance of differences between years (NIR)								Analysis of variance		Significance of differences between years (NIR)							
F	p	2005–2006	2006–2007	2007–2008	2008–2009	2009–2010	2010–2011	2011–2012	2012–2013	F	p	2005–2006	2006–2007	2007–2008	2008–2009	2009–2010	2010–2011	2011–2012	2012–2013
25.307	**<0.001**	**<0.001**	0.465	**<0.001**	**0.025**	0.550	**<0.001**	**<0.001**	0.116	24.792	**<0.001**	**0.007**	0.721	**0.002**	**<0.001**	**<0.001**	**0.007**	**<0.001**	0.131
Year	2005	2006	2007	2008	2009	2010	2011	2012	2013	Year	2005	2006	2007	2008	2009	2010	2011	2012	2013
mean	23.17	32.44	30.39	40.2	44.93	46.16	37.15	27.06	31.31	mean	90.74	124.72	129.44	164.92	207.94	173.41	148.74	103.14	122.28

Progressive motility [%]									
Analysis of variance		Significance of differences between years (NIR)							
F	p	2005–2006	2006–2007	2007–2008	2008–2009	2009–2010	2010–2011	2011–2012	2012–2013
4.747	**<0.001**	0.153	0.538	**<0.001**	**0.031**	0.687	0.623	**0.040**	0.858
Year	2005	2006	2007	2008	2009	2010	2011	2012	2013
mean	20.16	18.67	18	21.86	20.08	19.76	19.39	17.8	17.61

Morphologically normal spermatozoa [%] (WHO 1999)						Morphologically normal spermatozoa [%] (WHO 2010)				
Analysis of variance		Significance of differences between years (NIR)				Analysis of variance		Significance of differences between years (NIR))		
F	p	2005–2006	2006–2007	2007–2008	2008–2009	F	p	2010–2011	2011–2012	2012–2013
33.375	**<0.001**	**0.004**	**<0.001**	**0.001**	**<0.001**	87.698	**<0.001**	**<0.001**	**<0.001**	0.648
Year	2005	2006	2007	2008	2009	Year	2010	2011	2012	2013
Mean	36.80	33.20	26.78	30.55	26.03	Mean	24.53	16.97	10.63	9.87

Abbreviations: *F* – *F* test-*statistic;* p – statistical significance.

over the examined years, without a clear tendency, which was confirmed by analysis of variance (F=90.139; p<0.001), and statistically significant differences according to the NIR test concerned all the subsequent years, except for 2010/2011. The mean percentage of morphologically normal spermatozoa according to the WHO 2010 criteria increased among Ukrainians during the years of the study, which was observed based on the analysis of variance (F=14.23; p<0.001); however, a statistically significant difference according to the NIR test concerned only the years 2010/2011 (Tab. 2).

DISCUSSION

In the presented study, spermiograms were compared of men reporting for the first time to therapeutic centres due to infertility in the Lublin and in the Lvov regions, using at the beginning the WHO 1999 criteria, and subsequently the WHO 2010 criteria. Catanzariti et al. in their study noted that the changes from WHO 1999 to WHO 2010 criteria did not modify the interpretation of semen quality, because comparing the two classifications they demonstrated that there is a substantial agreement, considering the three parameters (count, motility and morphology) all together, and also considering each single parameter [7]. Therefore, in this study, progressive motility was analyzed (fast and slow together), as well as sperm concentration, not considering the changes in evaluation criteria according to the WHO, while with respect to sperm morphology the analysis concerned investigations acc. to 1999 and 2010 criteria separately.

While evaluating changes in sperm concentration, the total number of spermatozoa, and the percentage of spermatozoa which showed progressive motility in Poland and Ukraine, despite the fact that the results between individual years differed, no unequivocal tendency was observed towards changes of these parameters in a specified direction during the period 2005–2013. With respect to sperm concentration, this is in accordance with the results obtained by other Polish researchers Semczuk et al. conducted in the Lublin Region, and Pająk et al., as well as Olejek et al. who investigated the Silesian Region (Poland) [9, 10, 11]. Studies by Olejek et al. were retrospective, and covered 2,116 men who had not been treated before and reported to the Centre for Infertility Treatment during the period 1982–1997 [11]. Similar to the presented studies, these researchers did not observe any tendency towards changes in sperm motility which would be of a considerable clinical importance. The results of this study and the study by Semczuk et al., Pająk et al., as well as by Olejek et al., suggest that the situation is stable from the aspect of the amount of sperm produced and its motility among men in Poland [9, 10, 11].

While analyzing the percentage of the mean number of spermatozoa with normal morphology, a clear tendency was noted in the Lublin Region towards decrease in this parameter during the examined years according to the WHO 1999 as well as the 2010 criteria. Semczuk et al., and Pająk et al. in their studies made similar observations and noted a systematic increase in this parameter, whereas Olejek et al. did not discover any changes with respect to sperm morphology in their patients [9, 10, 11]. Undoubtedly, this evokes the need for further monitoring of changes in sperm morphology in larger study groups, and if the occurrence of this phenomenon is confirmed, seeking the causative agent.

Studies concerning other regions of the world conducted by Carlsen et al. in 1992 demonstrated that there has been a genuine decline in semen quality over the past 50 years [5]. These studies were based on the analysis of the results of investigations in various countries worldwide, and nearly a half of them concerned the United States. These studies were continued by Swan et al. [16]. In that analysis, they found significant declines in sperm concentration in the United States and Europe/Australia after controlling for abstinence time, age, percent of men with proven fertility, and specimen collection method. The declines in sperm concentration in the United States (approximately 1.5%/year) and Europe/Australia (approximately 3%/year) were somewhat greater than the average decline reported by Carlsen et al. (approximately 1%/year) [5, 16]. However, they found no decline in sperm concentration in non-Western countries, for which data were very limited, which seems to be in accordance with the results of the presented study, as well as the results obtained by Olejek et al., Pająk et al., and Semczuk et al. [10, 11, 12].

In many other countries, many investigators also performed retrospective studies and reported that semen quality had been declining in the past several decades (Sripada et al.;

Table 2. Changes in sperm parameters during the examined years among Ukrainians.

Sperm concentration [x10^6/ml]						Total sperm concentration [mln]					
Analysis of variance		Significance of differences between years (NIR)				Analysis of variance		Significance of differences between years (NIR)			
F	p	2009–2010	2010–2011	2011–2012	2012–2013	F	p	2009–2010	2010–2011	2011–2012	2012–2013
133.97	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.014**	123.96	**<0.001**	**<0.001**	**<0.001**	**<0.001**	0.062
Year	2009	2010	2011	2012	2013	Year	2009	2010	2011	2012	2013
Mean	55.02	20.33	48.16	18.26	24.66	Mean	195.33	72.09	156.23	61.62	79.25
Progressive motility [%]						Morphologically normal spermatozoa [%] (WHO 2010) [%]					
Analysis of variance		Significance of differences between years (NIR)				Analysis of variance		Significance of differences between years (NIR)			
F	p	2009–2010	2010–2011	2011–2012	2012–2013	F	p	2010–2011	2011–2012	2012–2013	
90.139	**<0.001**	**<0.001**	0.091	**<0.001**	**<0.001**	14.235	**<0.001**	**<0.001**	0.364	0.478	
Year	2009	2010	2011	2012	2013	Year	2010	2011	2012	2013	
Mean	24.15	35.75	33.81	16.83	27.55	Mean	21.25	22.42	25.50	26.32	

Abbreviations: *F – F* test-*statistic;* p – statistical significance.

Feki et al.; Rolland et al.) [17, 18, 19]. While several others reported that there were no significant changes in human semen quality (Rasmussen. et al.) [20]. Asian scientists also undertook attempts to analyze changes in sperm parameters on their continent. Liang et al. examined sperm parameters in 5,834 Chinese men with confirmed fertility from 14 provinces, during the period 1980–2005 [21]. They confirmed a decrease in the concentration of spermatozoa per ml over the period of 25 years; however, after 1996 no longer noticed any considerable decrease in concentration. Li et al discovered that the concentration and viability of sperm of students from a university in the Czengdu Province gradually decreased [22]. Nevertheless, Zhu et al. analyzed 36 reports and examined sperm parameters in 2,318 healthy Chinese men, and indicated that there was no considerable decrease concentration of spermatozoa within the 13 years examined (1985–1997) [23]. It may be noted that the reports concerning China, with different life conditions from those in Europe, do not provide an unequivocal answer concerning the occurrence of changes in sperm parameters in this country during the course of time.

While comparing the selected sperm parameters between men from Poland and Ukraine, no statistically significant differences were found in sperm concentration, while the total number of spermatozoa was high in Polish ejaculates. Similar tendencies were observed in the study carried out by Toft et al., where the mean sperm concentration among men from Poland was 59×10^6/ml, whereas in Ukraine – 56 10^6/ml, and the total number of spermatozoa was 190×10^6 in Warsaw, and 170×10^6 in Kharkov [24]. However, in the studies by Mocevic et al., the mean sperm concentration and the total number of spermatozoa was considerably higher in Poland (88.8×10^6/ml; 338.7), compared to Ukraine (69.6×10^6/ml; 233.2×10^6 [25]. The results of these studies and the results of the presented study suggest that the amount of produced spermatozoa is higher in Poland, compared to Ukraine.

The quality of semen evaluated based on sperm morphology and motility in the presented study suggested the presence of a better situation among men living in Ukraine. Mocevic et al., and Toft et al. in their studies also compared the mean percentage of progressive motility and morphology of spermatozoon in semen between these countries [24, 25]. The only difference observed by them with respect to these parameters concerned a higher motility in Ukrainian ejaculates in the study conducted by Mocevic et al., which is consistent with the results of the presented study [25].

The results obtained in the presented study and some researchers comparing semen between Poland and Ukraine suggest that there are differences in the selected parameters. It seems extremely difficult to distinguish factors responsible for the occurrence of this phenomenon, due to the simultaneous effect of many factors. Studies of this problem conducted to-date by various researchers consider, among others things, differences in selected health behaviours and different exposure to environmental factors.

Toft et al., while comparing the burden of tobacco smoking between these countries, found that in Ukraine 71% of the men examined were smokers, while in Poland – 30%, whereas alcohol consumption was higher among Polish respondents, which may affect spermatogenesis and the level of sex hormones [24]. Bojar et al. [26] reported in their study that patients from the Lublin Region who were smokers constituted 27.41% of the patients examined. Differences in smoking may also result from the surveyed people's place of living, since more smokers are found in rural areas, according to research by Sygit K.et al. [27]. The problem of smoking among couples treated for infertility in the Lublin Region is also highlighted in research by Wdowiak et al. [28]. Passive smoking results in the development of embryos of poorer quality and also – the newborn just after birth.

Consales et al. reported that the level of pituitary gonadotropins and sperm DNA fragmentation was similar among males in both countries; however, the level of testosterone was higher among Ukrainians [29]. Stronati et al. emphasized the lack of sea fish in the diet of Poles, compared to Ukrainians [30]. The results of studies by Toft et al., and Stronati et al. showed the occurrence of significant differences in indicate the occurrence of significant differences in health behaviours between these populations, and it may be expected that the number of these differences may be much greater and, undoubtedly, may cause the occurrence of differences in semen quality between countries [24, 30].

Gibb et al., Lech et al. and Marzec et al. found that in Ukraine and Poland, blood mercury concentrations have been low and stable over a period of at least 12 years. Nevertheless, global mercury emissions may be increasing because of the burning of coal and fuel oil, especially in Asia, and Ukraine may, to a greater extent, be exposed to the effect of this phenomenon as shown by the report of Arctic Monitoring and Assessment Programme (AMAP, 2004) [31, 32, 33, 34]. In the studies by Andreucci et al. it was found that the concentration of cadmium in blood serum of Poles is lower than in Ukrainians, while an opposite relationship concern the level of zinc [35]. Lenters et al. discovered that the concentration of lead, cadmium, and hexachlorobenzene was higher in sera of Ukrainians than Poles, whereas the opposite was noted with respect to the level of perfluorooctane sulfonic acid, perfluorodecanoic acid and perfluorooctanoic acid. Studies concerning the exposure to numerous environmental contaminants show that the Ukrainian and Polish populations are exposed in a different way to these hazardous factors, which may be the cause of the differences observed in some parameters of sperm [36].

Studies conducted on animals by Anway et al. and Fernández-González et al. also confirmed that environmental factors or health behaviours, by generating, among others, oxidative stress which may exert an epigenetic effect on the occurrence of changes in fertility of future generations [37, 38]. The abnormalities observed on animal models concerned a decrease in ovarian reserve and deterioration of semen parameters. Thus, it may be expected that any hazards exerting an effect on the ancestors of the males in the study might have also contributed to the shaping of the quality of their sperm. The determination of epigenetic effect on fertility of factors which exerted an effect 30 years ago and earlier, seems to be rather impossible to carry out today.

Today, the potential occurrence of differences in health behaviours or exposure to selected environmental factors will not provide an unequivocal answer to the questions arising during analysis of their effect on human fertility. However, this may delineate a direction in which further studies should be conducted in order to monitor the reproductive health of the population. In the future, this may create the chance to undertake at an appropriate time prophylactic actions in the area of exposure to hazardous factor, and the results obtained

in the presented study determine the age group of men to whom these actions should be addressed. The presented studies will be continued in the future, subject to a stable situation in both countries.

CONCLUSIONS

1. Mean sperm concentration of patients reporting for the first time for infertility treatment in the Lublin Region and Lvov Oblast did not differ.
2. The total mean number of spermatozoa produced by Poles was higher than in Ukrainians.
3. The quality of semen with respect to motility and sperm morphology was better in patients from Ukraine than from Poland.
4. Despite differences concerning concentration, amount of sperm produced and percentage of spermatozoa showing progressive motility which occurred between individual years, no unequivocal tendency was observed towards change of these parameters in a specified direction in both countries over the years examined.
5. Among men examined in the Lublin Region, a tendency was noted towards decrease in the percentage of morphologically normal spermatozoa in the ejaculate during the years examined, while among patients from the Lvov Region an opposite tendency was observed.

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