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## ORIGINAL ARTICLE

Sperm Biology

# Environmental exposure to cadmium but not lead is associated with decreased semen quality parameters: quality regionalism of sperm properties

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Environmental factors may negatively contribute to a progressive worsening of semen quality, and differences in semen quality may result from different environmental exposures (regional differences) or lifestyle differences. Heavy metals are factors with a confirmed negative influence on male fertility. Among them, lead and cadmium are commonly found in human surroundings. Thus, we analyzed semen parameters (according to the World Health Organization 2010 recommendations) and semen lead and cadmium concentrations in 188 men from two different regions in Poland, a typical agricultural area and an industrial area, in couples that had been diagnosed with infertility. The assays were performed using flameless electrothermal atomic absorption spectrometry. In the statistical analysis, regional comparisons and then taxonomic comparisons based on three parameters (age, semen concentration, and sperm morphology) were applied. We showed that more cadmium than lead accumulated in semen, a higher cadmium concentration was observed in semen obtained from men from the agricultural region, and better semen quality and lower cadmium concentrations were found in the semen of men from the industrial, more polluted region. We thus showed an existing regionalism in the sperm quality properties. However, semen parameters such as morphology and progressive and nonprogressive motility followed the same trends, regardless of the patient's age, region, or class. We could conclude that the environment has a minor impact on sperm morphology and progressive and nonprogressive motility and that other existing factors could have an indirect influence on semen quality.

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## INTRODUCTION

Male fertility is influenced by many external and internal factors.<sup>1</sup> Approximately 40%–90% of male infertility is attributed to disturbances in sperm production, often of unknown cause.<sup>2</sup> Sociodemographic factors are among those that have been widely investigated with respect to semen quality. It has been shown that disparities in semen parameters exist within European countries, between men in Baltic countries and those in other European countries, and in the US between men from the mid-west and from western states.<sup>3</sup> It has been suggested that differences in semen quality may result from different environmental exposures (regional differences) or lifestyles.<sup>3,4</sup> The role of environmental factors in male infertility may negatively contribute to a progressive worsening of semen quality. These factors may act on the male reproductive tract via several mechanisms. Some of these mechanisms are related to hormonal or antihormonal activity, and these factors may disturb testis function at different points throughout both development and adult life, including effects on Sertoli cells,

Leydig cells, and germ cells, where they can trigger mutagenesis.<sup>5</sup> However, it needs to be highlighted that investigating possible environmental influences on semen quality requires many potential confounding factors to be considered.

Heavy metals are elements with a confirmed negative influence on male fertility. Among them, lead and cadmium are commonly found in human surroundings. Thus, even environmental exposures may have an influence on human health. Lead is present in lead pipes, ceramic glazes, exterior plaster, fungicides, plants, and cigarettes.<sup>6</sup> Lead enters the respiratory tract and is also ingested via food and drink water.<sup>7–9</sup> The half-life of lead in blood is 35 days, and in bones, it may be as long as 20–30 years. Lead tends to accumulate in bone, central nervous system, and parenchymal organs. The mean concentration of lead in bones is higher for men than women.<sup>10</sup> The data about the influence of lead on semen parameters are confusing and often contradictory.<sup>6,11,12</sup> Similarly, cadmium is easily absorbed by plants and accumulates in the human body. The mining and fuel industries, as well as battery

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manufacturing, emit high amounts of cadmium into the environment.<sup>13</sup> Adults consume approximately 1 µg to 10 µg of cadmium via food intake daily.<sup>14–16</sup> The half-life of cadmium is 3–4 months in blood and 7–26 years in the kidneys. It accumulates in the liver, testes, ovaries, and placenta.<sup>17</sup> Animal experiments have revealed that cadmium in the testis causes atrophy, necrosis, and hydrops, resulting in vascular damage.<sup>18</sup> The International Agency for Research on Cancer classified cadmium as carcinogenic to humans.<sup>19</sup> The types of economic activity performed in an area, especially agricultural and industrial, can contribute to the local exposure to heavy metals. **Table 1** shows the three main economic activities in Poland.

The data in **Table 1** show that the highest concentration of employment in the agricultural sector is in the Lublin region (26.5%), which also has the lowest industrial rate (21.8%); in contrast, the most industrialized region is Silesia, with 39.7% of the population employed in this sector and only 2.6% in agriculture, the lowest agricultural rate.

The Lublin region is considered to be one of the environmentally cleanest regions in Poland, with two national parks, the Poleski and Roztoczański National Parks. Gas and dust pollution in this region is 5000.2 thousand tons and 2.0 thousand tons, respectively. The number of registered farmsteads in the Lublin region is 178 135 compared to 58 981 in the Silesia region. The lead and cadmium loads in the soil (1995–2010) ranged from 6.8 mg kg<sup>-1</sup> to 34.2 mg kg<sup>-1</sup> and from 0.15 mg kg<sup>-1</sup> to 1.04 mg kg<sup>-1</sup>, respectively.<sup>13</sup> In turn, Silesia is regarded as a leading industrial area with gas pollution of 38 759.8 thousand tons and dust pollution of 10.2 thousand tons, from particularly numerous factories.<sup>20</sup> The mean annual lead concentration is 5%–10% of the permissible contamination level; for cadmium, it is 12%–44%.<sup>21</sup> The lead and cadmium loads in the soil (1995–2010) were 10.7 mg kg<sup>-1</sup> to 1073.3 mg kg<sup>-1</sup> and 0.55 mg kg<sup>-1</sup> to 1.79 mg kg<sup>-1</sup>, respectively. The use of mineral fertilizers (phosphate fertilizers) reached 32.3 kg per hectare (ha) in the Lublin region and 23.1 kg per ha in the Silesia region in 2019.<sup>13</sup> Comparing this basic information, the environmental differences in the two regions are clear. In this study, we focused on possible patterns in semen quality in two environmentally different regions in Poland, *i.e.*, a typically rural and an industrial region.

## PARTICIPANTS AND METHODS

According to standard and taxonomic statistical analyses, we aimed to demonstrate the quality regionalism of the sperm properties, including

**Table 1: The percentages of regional economic activity in Poland (2013)<sup>20</sup>**

Province	Agricultural (%)	Industrial (%)	Service (%)
Dolnoslaskie	5.5	34.9	59.6
Kujawsko-Pomorskie	15.4	30.1	54.5
Lublin region	26.5	21.8	51.7
Lubuskie	8.7	33.2	58.2
Lodzkie	13.2	31.6	55.3
Malopolskie	11.9	31.3	56.8
Mazowieckie	11.0	22.2	66.8
Opolskie	11.9	36.2	51.9
Podkarpackie	17.9	30.4	51.8
Podlaskie	24.3	23.0	52.8
Pomorskie	7.1	31.0	61.9
Silesia region	2.6	39.7	57.7
Swietokrzyskie	21.8	27.8	50.4
Warmińsko-Mazurskie	12.7	30.8	56.5
Wielkopolskie	13.0	35.5	51.5
Zachodniopomorskie	8.0	29.2	62.8

morphological indicators and heavy metal (lead and cadmium) contamination.

## Study subjects

We enrolled men in couples diagnosed with infertility in the study. The duration of infertility in all cases was not shorter than 2 years. Semen analysis is a basic procedure in the process of infertility diagnosis. As further diagnostic steps revealed evident female factors, 188 men in total, of which 91 from the Lublin agricultural province (Group Agricultural [A]) and 97 from the Upper Silesia industrial province (Group Industrial [I]), were retained in the survey. The exclusion criteria for the study participants were chronic diseases, varicocele, and known occupational exposure to heavy metals. All patients gave their informed consent to participate in the investigation. The study was approved by the Local Ethical Committee of the Medical University of Silesia, Katowice, Poland (KNW/0022/KB1/I/13/09).

## Semen analysis

The seminological analyses were performed at the Demeter Laboratory of the Antrum Medical Center in Silesia and at Ab Ovo in Lublin, both of which are certified and undergo annual quality audits.

The specimens (one sample from each man) were collected after 3–5 days of sexual abstinence. The semen examination was performed according to the World Health Organization 2010 recommendations.<sup>22</sup> The remaining part of the samples was centrifuged (3000g, 10 min; Centrifuge MPW-56, MPW Medical Instruments, Warszawa, Poland), and the supernatants sealed tightly in cryovials were stored in liquid nitrogen for later analysis.

## Measurement of cadmium and lead concentrations

The measurements of lead and cadmium concentrations in the seminal plasma were performed at the Central Laboratory of Toxicology in Miasteczko Śląskie, Poland. The assays were performed using flameless electrothermal atomic absorption spectrometry (Unicam iCE 3000, Thermo Fisher Scientific, Waltham, MA, USA). Lamp carousels have six data-coded positions, each with its own independent power supply which is modulated at 200/240 Hz. Auto-alignment of the lamp carousel ensures maximum light throughput deuterium discharge lamps are used for background correction purposes. The radiation intensity emitted by these lamps decreases significantly with increasing wavelength so that they can be only used in the wavelength range between 190 and about 320 nm. The lead concentration in the samples was determined at a wavelength of 283.3 nm as well in a reference solution concentration 29.0 (about scope: 27.0–31.0) µg l<sup>-1</sup> (Seronorm™, Nycomed Pharma, Oslo, Norway). The cadmium concentration was determined at a wavelength of 228.8 nm. Reference solutions (Seronorm™, Nycomed Pharma), 65% nitric acid (Merck KGaA, Darmstadt, Germany), deionized water, and reference materials for intralaboratory control (Seronorm™, Nycomed Pharma) were used. The precision as indicated by relative standard deviation (repeatability) was 0.8 for lead and 5.9 for cadmium; the reproducibility was 2.68 and 4.97, respectively. The detection limit was 0.14 µg l<sup>-1</sup> for lead and 0.10 µg l<sup>-1</sup> for cadmium.

## Statistical analyses

The statistical computing program R Core Team, 2020 (R Foundation for Statistical Computing, Vienna, Austria) was applied as the analytical method.<sup>23</sup> First, Student's *t*-test (independent samples) and Wilcoxon's test were used to compare the collected indicators (semen parameters) and heavy metal (lead and cadmium) contamination between Group A and Group I.



Second, to determine the possible regional effect on semen quality, taxonomic analysis was applied. Finally, to quantify the difference between the regional and taxonomic studies, we carried out a meta-analysis (systematic review and synthesis) to combine the results.  $P < 0.05$  was considered statistically significant.

## RESULTS

The results of the first statistical analysis, Student's *t*-test (independent samples), and Wilcoxon's test to compare Group A and Group I are shown in **Table 2**. In the regional analysis, the differences were statistically significant ( $P < 0.05$ ) in all the sperm morphological indicators examined (**Table 2**, panel A), excluding sperm volume and lead concentration, which was higher in Group I, but the difference was borderline ( $P < 0.1$ ). Surprisingly, the cadmium concentration in semen was higher in Group A. Then, to determine a possible effect of the heavy metals on the semen quality, taxonomic statistics were performed (analogous studies were performed in other studies).<sup>24,25</sup> In this approach, the patients' sperm count per ml, percentage of normal morphology, and age were used to classify the patients because there are many studies raising the issue of heavy metal accumulation related to age; moreover, semen concentration and morphology are important fertility parameters.<sup>26–28</sup> The use of taxonomic statistics enabled us to distinguish 188 analyzed men into two new groups of patients (named Class 1 and Class 2). The results as a classification tree (dendrogram) are presented in **Figure 1**.

In the dendrogram, two classes of patients (Class 1 and Class 2) could be established with respect to the analyzed clinical factors (**Figure 1**). Then, the same tests as in the regional analysis were used to compare the patients allocated to these two classes (**Table 2**, panel B). In the taxonomic analysis, all of the differences were statistically significant ( $P < 0.05$ ), excluding leukocytes and lead. In addition, it was found that nearly two-thirds of the Lublin (agricultural) population belonged to Class 2; similarly, approximately two-thirds of Silesians (industrial) belonged to Class 1.

Finally, to quantify the standardized mean differences (SMDs) between the regional and taxonomic data, a meta-analysis (systematic review and synthesis) was carried out to combine the results as shown in **Table 2** (panels A and B).<sup>29</sup> Since the heterogeneity for all of the studies was significant ( $P < 0.0001$ ), it was assumed that some variation in the results was due to the role of chance, *i.e.*, random variation. Therefore, in the statistical analysis, a random-effects model was applied.<sup>30</sup> The results of the meta-analysis are graphically displayed in a forest plot in **Figure 2**.

According to the results shown in **Figure 2**, it can be established that the largest differences between regional and taxonomic classifications of patients appeared in the percentages of correct morphology, progressive motility, nonprogressive motility, and age. However, based on the obtained *P* values for particular clinical factors (**Table 2**, panel C) and the model of SMD (0.0042, 95% confidence interval [CI]:  $-0.1312$ – $0.1396$ ;  $P = 0.9515$ ; **Figure 2**), it can be stated with a high degree of certainty that Group I almost equals to Class 1 and Group A almost equals to Class 2.

## DISCUSSION

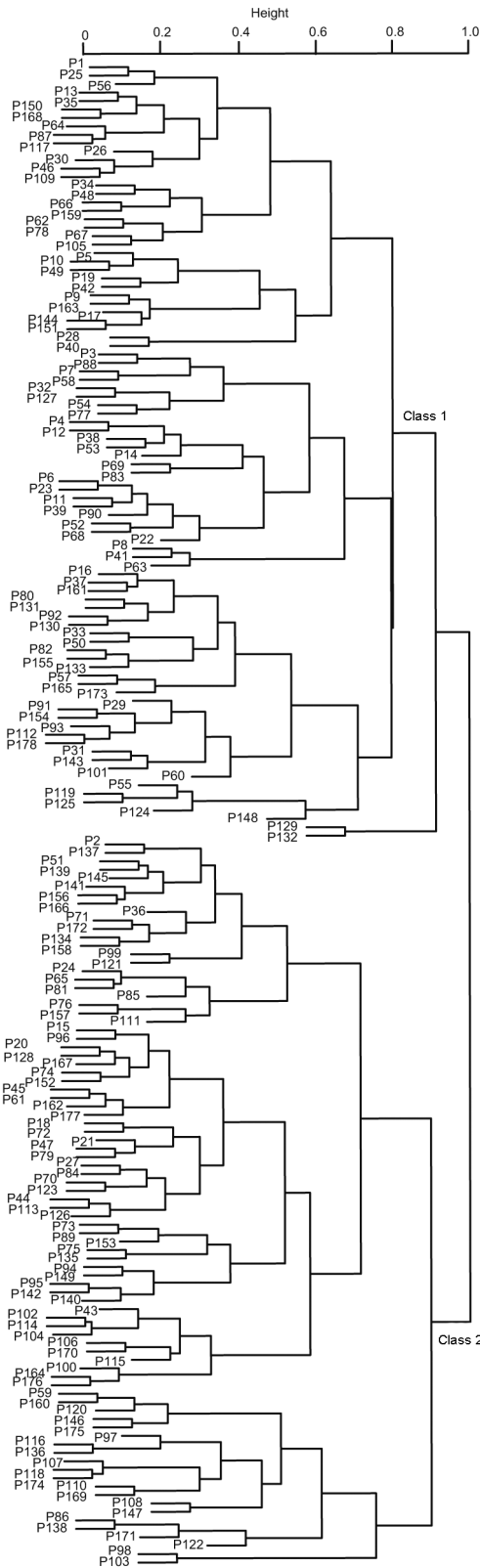
Our research concerning semen quality and concentrations of heavy metals (cadmium and lead) was performed in two environmentally distinct regions in Poland. Our initial results were unexpected. Although 70% of the patients' semen parameters were within normal ranges, we found that the sperm parameters of patients from the industrial region were better than those of patients from the agricultural region. Geographical affiliation with the agricultural region was related to decreased sperm concentration, worse morphology, and a decreased percentage of immotile sperm. In addition, we observed a higher cadmium concentration in the semen of men living in the agricultural region. The results of this first analysis were statistically significant.

There is no broad agreement concerning the influence of cadmium on semen parameters. Meeker *et al.*<sup>31</sup> showed no correlation between the level of cadmium in the blood and semen quality. Age was the only positive confounder correlated with seminal plasma cadmium in studies by Benoff *et al.*<sup>32</sup> In our study, no correlation between age and cadmium concentration in semen was observed, and the age of the patients from the analyzed regions was similar. In the study of Pant *et al.*,<sup>33</sup> cadmium correlated negatively with motility and sperm concentration. Our studies confirm these findings. In *in vitro* studies, it was reported that, even with low cadmium concentrations, testis germ cell survivability is changed, and apoptosis processes are induced in a dose- and time-dependent manner.<sup>34,35</sup> Moreover, cadmium is thought to be an endocrine disruptor and metalloestrogen.<sup>36</sup> The mechanisms of cadmium toxicity on the endothelium are complex and include effects on endothelial mediator activity, endothelial cell proliferation, angiogenesis, coagulation, and the fibrinolysis system.<sup>37</sup> Studies on animals show that cadmium causes decreases in testicle weight, in the amount of testosterone produced, and in sperm count

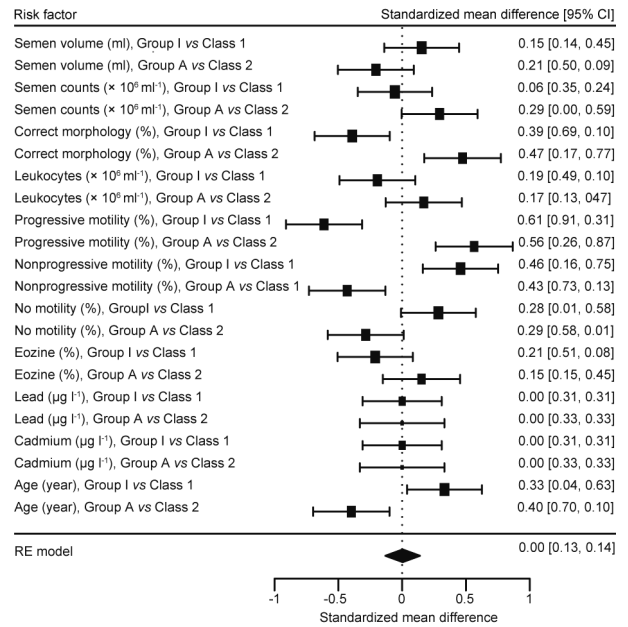
**Table 2: Clinical characteristics of patients as the mean±s.d. and associated *P* values**

Clinical factor	Panel A				<i>P</i>	Panel B				Panel C, <i>P</i>	
	Group A	<i>n</i> <sup>a</sup>	Group I	<i>n</i> <sup>b</sup>		Class 1	<i>n</i> <sup>c</sup>	Class 2	<i>n</i> <sup>d</sup>		
Semen volume (ml), mean±s.d.	4.1±1.4	89	4.0±1.4	88	0.6313	3.8±1.2	91	4.4±1.5	86	0.007	0.886
Sperm counts (×10 <sup>6</sup> ml <sup>-1</sup> ), mean±s.d.	25.7±22.2	89	71.0±74.1	89	<0.0001	75.1±70.7	92	19.8±17.5	86	<0.0001	0.5033
% Correct, mean±s.d.	7.7±6.9	89	13.6±6.1	89	<0.0001	15.9±5.6	92	5.1±3.4	86	<0.0001	0.9255
Leukocytes (×10 <sup>6</sup> ml <sup>-1</sup> ), mean±s.d.	0.25±0.39	87	0.08±0.17	87	0.0004	0.13±0.32	88	0.19±0.31	86	0.2219	0.9448
Progressive motility (%), mean±s.d.	32.4±13.1	89	29.0±11.3	89	0.0654	35.7±10.5	92	25.3±11.9	86	<0.0001	0.9673
Non-progressive motility (%), mean±s.d.	8.7±5.3	89	21.9±5.6	89	<0.0001	18.8±7.7	92	11.6±7.9	86	<0.0001	0.9753
No motility (%), mean±s.d.	58.9±14.4	89	49.2±14.4	89	<0.0001	45.6±10.6	92	63±14.2	86	<0.0001	0.9998
Eozine (%), mean±s.d.	66.7±15.2	84	72.6±11.9	89	0.0058	74.8±8.6	89	64.3±16.3	84	<0.0001	0.8622
Lead (μg l <sup>-1</sup> ), mean±s.d.	0.39±0.25	71	0.46±0.23	79	0.0862	0.46±0.23	81	0.39±0.25	69	0.1237	1
Cadmium (μg l <sup>-1</sup> ), mean±s.d.	0.32±0.11	71	0.26±0.11	79	0.001	0.26±0.10	81	0.32±0.12	69	0.0048	1
Age (year), mean±s.d.	33.3±3.9	89	34.9±4.9	89	0.0177	33.3±4.7	92	34.9±4.1	86	0.022	0.929
Region	NA		NA		NA	Group I=65	92	Group A=66	86	<0.0001	NA

<sup>a</sup>Patients number of Group A; <sup>b</sup>patients number of Group I; <sup>c</sup>patients number of Class 1; <sup>d</sup>patients number of Class 2. Panel A: regional comparison; Panel B: taxonomic comparison; Panel C: regional/taxonomic comparison; Group A: agricultural region; Group I: industrial region; NA: not available; s.d.: standard deviation



**Figure 1:** Patient classification tree according to sperm count per ml, percentage of correct morphology, and age ( $n=178$ ). Individual patients (coded by “P” numbers) from top to bottom are hierarchically aggregated in separate classes, ultimately representing individual leaves in the dendrogram; based on this, groups of patients can be distinguished.



**Figure 2:** Meta-analysis between regional and taxonomic analyses of patients in terms of available clinical factors of semen. CI: confidence interval.

and activity.<sup>38</sup> As shown by Farag *et al.*,<sup>39</sup> three-month administration of cadmium impaired dysfunction of the prostate gland and decreased secretion capacity. Data on the influence of heavy metals on the male reproductive system are often derived from animal studies. It is not advised to generalize all of the results from those studies to humans; however, the value of these results cannot be ignored. The duration of exposure is also significant.<sup>40,41</sup> Cadmium negatively influences sperm maturation and morphology, which was also observed in our study.

The lead concentration was slightly higher in the industrial region, but the difference between the two regions was not significant. However, we noted that, when the mean seminal plasma lead concentration was higher than  $\geq 0.5 \mu\text{g dl}^{-1}$ , a decreased sperm concentration ( $< 10 \times 10^6 \text{ ml}^{-1}$ ;  $P = 0.02860$ ) was observed, regardless of the patients’ region (data not shown). Xu *et al.*<sup>42</sup> and Pant *et al.*<sup>33</sup> found no influence of lead levels on semen quality (motility, concentration, morphology, volume, and vitality). A study by Telisman *et al.*<sup>43</sup> revealed that a higher lead concentration is associated with decreased sperm motility, while other studies have stated that an increased lead concentration is not associated with any changes in male endocrine system function; however, Mendiola *et al.*<sup>44</sup> revealed a positive correlation between the nonmotile sperm percentage and the lead and cadmium concentrations in the semen and blood. In our study, lead had no influence on sperm motility. In accordance with Xu *et al.*,<sup>42</sup> it seems that lead probably had no influence on semen quality if the concentration in seminal fluid did not exceed  $10 \mu\text{g l}^{-1}$ . The cutoff value is commonly established at  $40 \mu\text{g l}^{-1}$  for lead concentration in blood. Above this value, the deterioration in sperm motility and a toxic influence on the hypothalamo–pituitary–testis axis were observed.

Two-step statistical analysis was performed. Since no linear cause–effect relationships were found in the two regions in terms of semen parameters via either nonparametric correlation or multivariate regression, the next analysis was based on a taxonomic method. The preliminary intention was to investigate whether the influence of heavy metals on semen quality may be independent of region. Taxonomic analysis was based on

three parameters: patient age, semen concentration, and morphology. We chose patient age because there is substantial research related to the issue of heavy metal accumulation related to age, semen concentration, and morphology, which are important fertility parameters.<sup>26–28</sup>

Taxonomic analysis (**Figure 1**) revealed that, among all 188 patients, two groups (classes) existed, but surprisingly, they were not homogenous in terms of the patients' geographic origin (missing information was imputed to be caused by randomness). Class 1 included 91 patients, of whom men from the industrial region were 59 cases (64.8%) and 32 (35.2%) were from the agricultural region. In Class 2 (86 patients), 37.2% of patients represented the industrial region (32 cases) and 62.8% represented the agricultural region (54 cases). The taxonomic comparison (**Table 2**, panel B) partially coincided with the regional comparison (**Table 2**, panel A), where the place of residence (region) was an "allocation factor." Generally, Class 1 was dominated by younger men from the industrial region (mean age: 34.2 years). Approximately one-third of this group consisted of men from the agricultural region (mean age: 31.8 years). Class 2 consisted mainly of patients from the agricultural region who were older (mean age: 34.2 years) than those men from the agricultural region who (after the second analysis) were allocated in Class 1. One-third of Class 2 consisted of older men from the industrial region (mean age: 36.3 years). Comparing the results (**Table 2**), we concluded that division into groups based solely on patient region (panel A, regional statistics) might not lead to the correct interpretation. We applied the statistics shown in panel B (clinical statistics) to reveal other possible relationships between the patients' semen quality parameters and heavy metals. Cadmium and lead concentrations did not differ between the two statistical panels; however, only for cadmium were the correlations of its level with semen parameters strongly statistically significant. It might then be assumed that clinical and regional characteristics make roughly equal contributions (*i.e.*, panel B and panel A). Moreover, in our study, we aimed to evaluate the differences in semen parameters and two heavy metals (cadmium and lead) between two environmentally different regions, but not as far a distance as Glazer *et al.*<sup>3</sup> showed for men from distant regions in one country. In addition, Nordkap *et al.*<sup>35</sup> showed significant differences in semen quality between men from four different European cities. These results indicate that, even among geographically closer groups representing different regions, clinical differences are present.

It is a matter of question why the new groups that emerged from taxonomic analysis of the 188 patients, namely, Class 1 and Class 2, repeated the pattern of semen parameters in the manner observed in patients assigned strictly by their place of living (first, regional analysis). We could conclude that the environment has only a minor impact on these three parameters.

Factors that probably have an impact on semen quality in men with environmental exposure are not easily identified. Lifestyle factors include the amount and kind of cigarettes smoked and alcohol consumption (similar in both groups, data not shown), sport activity, vitamin and antioxidant intake, diet, and absorption of contaminated soil and dust. Despite the more common use of phosphate fertilizers in agricultural regions (32.3 kg per ha vs 23.1 kg per ha in industrial regions, data from 2019<sup>20</sup>), the cadmium content in soil is higher in industrial regions (4.958 mg kg<sup>-1</sup> vs 0.158 mg kg<sup>-1</sup> in agricultural regions).<sup>20</sup> It is possible that the diets of men in the industrial region (where the cadmium concentration was lower) contained more antioxidants, or perhaps diet supplement intake was higher.<sup>46,47</sup> Conversely, other sources of cadmium in the agricultural region cannot be excluded.

There are a few other limitations to our study. First, semen analysis was performed at two different laboratories. Both of these laboratories

conduct tests in accordance with WHO recommendations. However, as pointed out by Leushuis *et al.*,<sup>48</sup> there is possible interlaboratory variability, especially for sperm morphology and concentration. Semen parameters were analyzed from one sample (in two regional laboratories), whereas a second sample was used for evaluation of heavy metals concentration (Central Laboratory of Toxicology). We are aware that an increased number of analyzed samples, especially within appropriate time intervals, would increase the specificity of the results. Repeat confirmatory tests should be performed 3 months after the initial analysis, as this is the time necessary for the whole cycle of spermatozoa formation.<sup>48</sup>

The next issue is the number of patients enrolled in the study. This results from the fact that we included only men whose partners had been diagnosed with female factor infertility. As female factor infertility was established, semen analysis among healthy male partners was not repeated. We did not assay lead and cadmium concentrations in patient serum. The serum concentrations play a crucial role in occupational exposure, and our goal was to investigate lead and cadmium in seminal plasma in environmentally exposed men, where these metals could be factors causing harmful effects on spermatozoa via free radicals.<sup>49</sup>

To summarize, our study showed a higher cadmium concentration in the agricultural region, and it seems that regional factors strongly influence semen quality. Interestingly, the men, who presented better semen quality and lower cadmium concentration despite living in an environment more polluted by heavy metals, tended to be older (however, without statistical significance) which is in opposition to the fact that cadmium accumulates with age. This phenomenon could be further investigated in terms of adaptive mechanisms. Another issue that deserves explanation is the observation that cadmium rather than lead accumulated in semen. We also observed that, similar to age, semen parameters such as morphology and progressive and nonprogressive motility followed the same patterns regardless of the patient region or class. This would mean that other factors could have a strong influence on men's reproductive health, indirectly causing higher cadmium concentrations in semen.

## AUTHOR CONTRIBUTIONS

KOW carried study design, data analysis, and manuscript writing. AT carried manuscript writing and statistical analysis. AK performed measurements of lead and cadmium concentrations in the seminal plasma. AW was involved in patient management and semen analysis. SH carried patient management and semen analysis, study coordination, manuscript drafting, and supervision. All authors read and approved the final manuscript.

## COMPETING INTERESTS

All authors declared no competing interests.

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