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Age-Dependence of Some Trace Element Concentrations and their Ratios in Human Prostatic Fluid

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Abstract

The effect of age on Br, Fe, Rb, Sr, and Zn concentrations as well as on Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr content ratios in human prostatic fluid was investigated by ^{109}Cd radionuclide-induced energy dispersive X-ray fluorescent microanalysis. Specimens of expressed prostatic fluid were obtained from 51 men (mean age 51 years, range 18-82 years) with apparently normal prostates using standard rectal massage procedure. Mean values (M ± SEM) for concentration of trace elements ($mg \cdot L^{-1}$) in human prostate fluid were: Br 3.62±0.58, Fe 9.04±1.21, Rb 1.10±0.08, Sr 1.19±0.14, and Zn 573±28. Mean values for ratios of trace elements in human prostate fluid were: Zn/Br 523±103, Zn/Fe 105±16, Zn/Rb 661±63, and Zn/Sr 719±95. An age-related increase in Zn content and decrease in Br and Fe concentration was found. Moreover, the strongly pronounced increase in Zn/Br and Zn/Fe ratios was also observed.

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Introduction

One of the main functions of prostate gland is a production, storage and excretion of prostatic fluid with extremely high concentration of Zn and some other trace elements (TE) and electrolytes.[1,2] During ejaculation, the liquid that is released (sperm or ejaculate) has about 30% of its content contributed to by the prostatic fluid. Thus, the prostatic fluid very strong effects on the chemical element composition of sperm. Role of prostatic fluid is very important because it basically helps in increasing the chances of impregnation.

There is a growing number of evidence indicating that advanced male age can affect fertility.[3-5] Numerous studies have investigated age-based alterations in semen traits, including such parameters as semen volume, sperm concentration, total sperm count, morphology, total motility, progressive motility and DNA fragmentation and some others. In most of these studies researches tried to determine whether age thresholds for parameters of semen quality exist. It was found that many measured parameters of ejaculates begin to change after 40 years of age. Forty years of age is also very significant thresholds for incidence of such prostatic diseases as being benign prostatic hypertrophy (BPH), and prostatic carcinoma (PCa). For example, it was reported that the risk of having PCa drastically increase with age, being three orders of magnitude higher for the age group 40–79 years than for those younger than 39 years.[6,7]

Experimental and epidemiological studies have reported the effects of some TE in ejaculate on male reproductive function.[8-10] Moreover, it was shown that Zn and Ca excess in prostatic fluid is one of the main factors in the etiology of BPH and PCa.[11,12] Thus, it seems fair to suppose that changes of TE contents in prostatic fluid after 40 years of age play a role in the male infertility and the pathophysiology of the prostate. However, at our knowledge there are no studies regarding the effect of age on contents and relationships of TE in prostatic fluid with the exception of Zn.

The primary purpose of this study was to determine reliable values of the Br, Fe, Rb, Sr, and Zn concentration in the intact prostatic fluids of apparently healthy subjects ranging from young adult males to elderly persons using ^{109}Cd induced energy dispersive X-ray fluorescent microanalysis (^{109}Cd EDXRF) developed by us.[13] The second aim was to calculate Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in all samples of prostatic fluid. The third aim was to compare the obtained results with reported data for TE concentrations and Zn/TE content ratios in prostatic fluid. The forth aim was to compare the TE concentrations and Zn/TE content ratios in prostatic fluid samples of age group 2 (aged 41 to 82 years), with those of group 1 (aged 18 to 40 years). The final aim was to check the correlations between age and all investigated parameters of prostatic fluid.

Materials and Methods

Samples

Specimens of expressed prostatic fluid (EPF) were obtained from 51 men (mean age 51 years, range 18-82 years) with apparently normal prostates by qualified urologist in the Urological Department of the Medical Radiological Research Centre using standard rectal massage procedure. Subjects were asked to abstain from sexual intercourse for 3 days preceding the procedure. The cytological and bacteriological investigations were used to control the norm conformity of prostatic fluid samples chosen for ^{109}Cd EDXRF.

The Ethics Committee of the Medical Radiological Research Centre approved the study, and participants gave their informed consent. All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Sample Preparation

Specimens of EPF were obtained in sterile containers which were appropriately labeled. Twenty μL (microliters) of fluid were taken by micropipette from every specimen for TE analysis, while the rest of the fluid was used for cytological and bacteriological investigations. The chosen 20 μL of the EPF was dropped on 11.3 mm diameter disk made of thin, ash-free filter papers fixed on the Scotch tape pieces and dried in a desiccator at room temperature. Then the dried sample was covered with 4 mm Dacron film and centrally pulled onto a Plexiglas cylindrical frame.

Instrumentation and Method

The facility for radionuclide-induced EDXRF included an annular ^{109}Cd source with an activity of 2.56 GBq, Si(Li) detector and portable multi-channel analyzer combined with a PC. Its resolution was 270 eV at the 6.4 keV line. The facility functioned as follows. Photons with a 22.1 keV ^{109}Cd energy are sent to the surface of a specimen analyzed inducing the fluorescence K_a X-rays of trace elements. The fluorescence irradiation got the detector through a 10 mm diameter to be recorded.

The duration of the Br, Fe, Rb, Sr, and Zn concentration measurement in one EPF sample was 60 min. The intensity of K_a-line of Br, Fe, Rb, Sr, and Zn for samples and standards was estimated on calculation basis of the total area of the corresponding photopeak in the spectra. The trace element concentration was calculated by the relative way of comparing between intensities of K_a-lines for samples and standards. To determine concentration of the elements by comparison with a known standard, aliquots of solutions of commercial, chemically pure compounds were used for a device calibration.[14] The standard samples for calibration were prepared in the same way as the samples of prostatic fluid. Details of the analytical method and procedures used here for sample preparation were presented in our earlier publications concerning the chemical elements of human prostatic fluid and tissue.[13,15-18]

Certified Reference Material

Because there were no available liquid Certified Reference Material (CRM) ten sub-samples of the powdery CRM produced by the International Atomic Energy Agency (IAEA) - CRM IAEA H-4 (animal muscle) were analyzed to estimate the precision and accuracy of results. Details of the procedures used for CRM sample preparation and measurement were presented in our earlier publication.[13,15-18]

Computer Programs and Statistic

Using the Microsoft Office Excel software to provide a summary of statistical results, the arithmetic mean, standard deviation, standard error of mean, minimum and maximum values, median, percentiles with 0.025 and 0.975 levels were calculated for all the TE concentrations and Zn/TE ratios obtained. The difference in the results between two age groups was evaluated by parametric Student's *t*-test and non-parametric Wilcoxon-Mann-Whitney *U*-test. Values of $p<0.05$ were considered to be statistically significant. For the estimation of the Pearson correlation coefficient between age and TE concentration, between age and Zn/TE ratio, as well as for the construction of "individual data sets for TE concentrations or Zn/TE ratios versus age" diagrams the Microsoft Office Excel software was also used.

Results

Table 1 depicts our data for Br, Fe, Rb, Sr, and Zn mass fractions in ten sub-samples of CRM IAEA H-4 (animal muscle) and the certified values of this CRM. Of four TE (Br, Fe, Rb, and Zn) with certified values for the CRM we determined contents of all certified elements (Table 1). Mean values (M±SD) for Br, Fe, Rb, and Zn were in the range of 95% confidence interval. Good agreement of the TE contents analyzed by ^{109}Cd radionuclide-induced EDXRF with the certified data of CRM IAEA H-4 (Table 1) indicate an acceptable accuracy of the results obtained in the study of the prostatic fluid presented in Tables 2-5.

Table 2 presents certain statistical parameters (arithmetic mean, standard deviation, standard error of mean, minimal and maximal values, median, percentiles with 0.025 and 0.975 levels) of the Br, Fe, Rb, Sr, and Zn concentrations as well as of the Zn/Br, Zn/Fe, Zn/Rb, Zn/Sr ratios in EPF of apparently healthy men.

The comparison of our results with published data for TE concentrations in the normal human prostatic fluid[15,19-27] is shown in Table 3.

To estimate the effect of age on the TE concentrations and Zn/TE ratios in the EPF we examined two age groups: group 1 (aged 18 to 40 years, Mean=27.5 years) and group 2 (aged 41 to 82 years, Mean =59.1 years) (Table 4).

Calculated correlation coefficients between age and TE concentration as well as between age and Zn/TE ratios in the prostatic fluid are collected in Table 5.

Figure 1 depicts constructed "individual data sets for TE concentrations versus age" or "individual data sets for Zn/TE ratios versus age" diagrams with lines of trend. In our study the best fit in the proportion

Table 1. EDXRF data of Br, Fe, Rb, Sr, and Zn contents in the IAEA H-4 (animal muscle) reference material compared to certified values (mg/kg, dry mass basis)

Element	Certified values			This work results
	M	95% confidence interval	Type	M±SD
Fe	49	47 - 51	C	48±9
Zn	86	83 - 90	C	90±5
Br	4.1	3.5 – 4.7	C	5.0±1.2
Rb	18	17 - 20	C	22±4
Sr	0.1	-	N	<1

M – arithmetical mean, SD – standard deviation, C- certified values, N – non-certified values.

Table 2. Some basic statistical parameters of the Br, Fe, Rb, Sr, and Zn concentration (mg/L) and also Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio in human prostate fluid

Element or ratio	M	SD	SEM	Min	Max	Median	Per. 0.025	Per. 0.975
Br	3.62	3.26	0.58	0.49	10.0	1.63	0.498	9.16
Fe	9.04	7.28	1.21	1.27	39.8	7.84	1.29	21.3
Rb	1.10	0.51	0.08	0.38	2.45	1.03	0.41	2.36
Sr	1.19	0.79	0.14	0.036	3.44	1.18	0.037	3.16
Zn	573	202	28	253	948	552	260	941
Zn/Br	523	582	103	32.0	1882	246	40	1882
Zn/Fe	105	92	16	13.0	343	67.0	18.0	343
Zn/Rb	661	385	63	119	1612	536	214	1608
Zn/Sr	719	519	95	155	2321	602	169	1980

M - arithmetic mean, SD – standard deviation, SEM – standard error of mean, Min – inimum value, Max – maximum value, Per. 0.025 – percentile with 0.025 level, Per. 0.975 – percentile with 0.975 level.

Table 3. Median, minimum and maximum value of means of Br, Fe, Rb, Sr, and Zn concentration (mg/L) and also Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio in human prostatic fluid according to data from the literature

Element or ratio	Published data [Reference]			This work results
	Median of means (n)*	Minimum of means M or M±SD, (n)**	Maximum of means M±SD, (n)**	M±SD
Br	-	-	-	3.62±3.26
Fe	-	-	-	9.04±7.28
Rb	2.26 (1)	1.11±0.57 (15) [23]	2.35±1.85 (11) [23]	1.10±0.51
Sr	-	-	-	1.19±0.79
Zn	453 (19)	47.1(-) [21]	9870±10130 (11) [25]	573±202
Zn/Br	-	-	-	523±582
Zn/Fe	-	-	-	105±92
Zn/Rb	-	-	-	661±385
Zn/Sr	-	-	-	719±519

M - arithmetic mean, SD – standard deviation, (n)* – number of all references, (n)** - number of samples.

Table 4. Effect of age on mean values (M±SEM) of the Br, Fe, Rb, Sr, and Zn concentration (mg/L) and also Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio in human prostate fluid

Element or ratio	Age groups				Ratios
	Group I 18-40 year (M=27.5) n=13	Group II 41-82 year (M=59.1) n=38	Student's t-test $p\leq$	*U*-test* *p*	Group II to group I
Br	6.35±1.17	2.86±0.59	**0.025**	**<0.01**	0.450
Fe	12.1±1.9	8.29±1.42	0.127	>0.05	0.685
Rb	0.91±0.15	1.16±0.10	0.195	>0.05	1.27
Sr	0.87±0.21	1.27±0.17	0.161	>0.05	1.46
Zn	501±47	598±34	0.108	>0.05	1.19
Zn/Br	111±28	639±122	**0.00026**	**<0.01**	5.76
Zn/Fe	47±7	120±19	**0.00098**	**<0.01**	2.55
Zn/Rb	748±157	637±69	0.534	>0.05	0.852
Zn/Sr	665±106	733±116	0.670	>0.05	1.10

M – arithmetic mean, SEM – standard error of mean, *Wilcoxon-Mann-Whitney *U*-test, **bold** – significant difference ($p\leq0.05$)

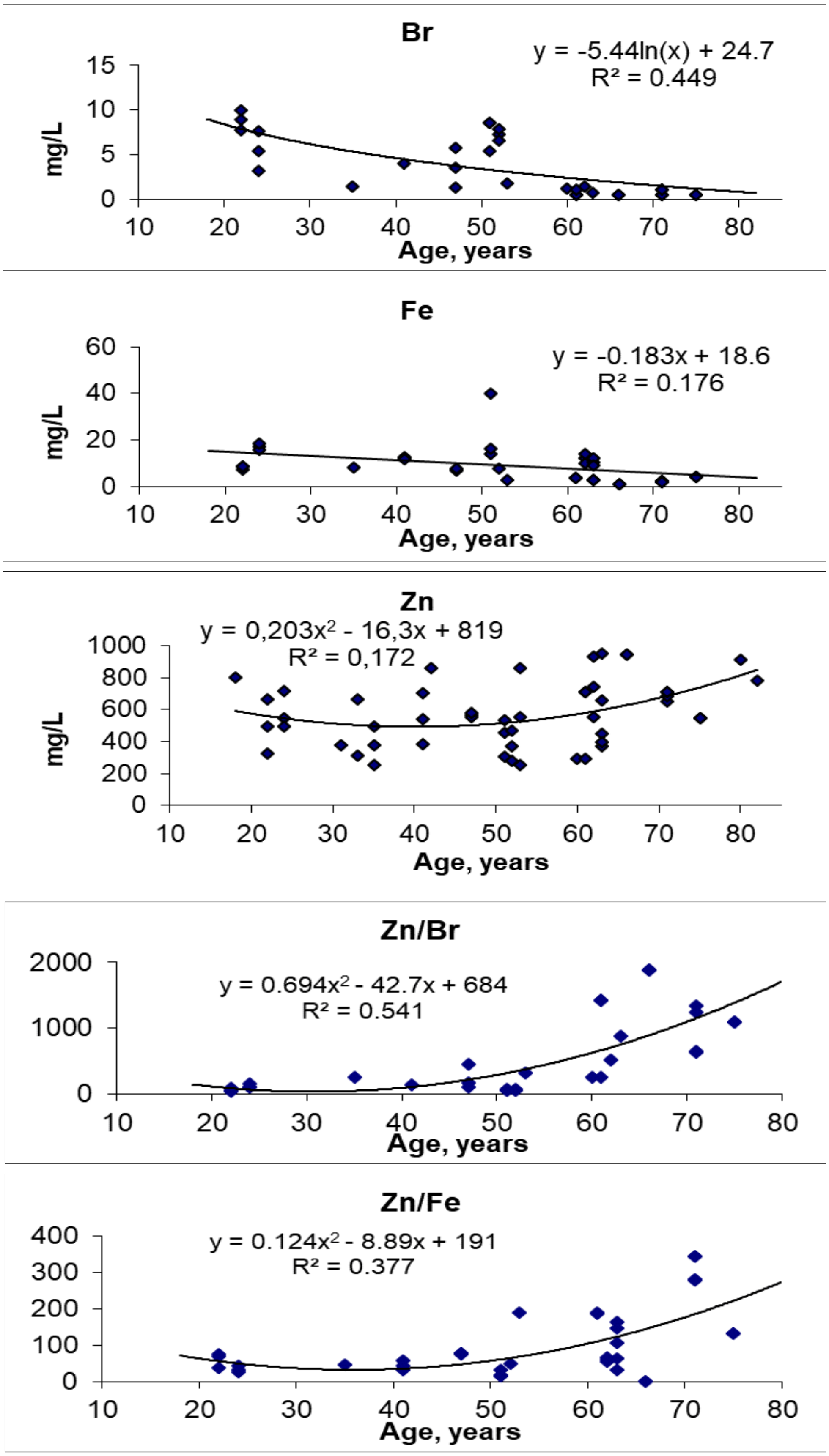

Figure 1. Data sets of individual concentrations of Br, Fe, and Zn as well as Zn/Br and Zn/Fe concentration ratio in prostatic fluid of healthy men and trend of these parameters with age

Table 5. Correlations between age and Br, Fe, Rb, Sr, and Zn concentration (mg/L) as well as Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr concentration ratio in human prostate fluid (r – coefficient of correlation)

Element or ratio	Br	Fe	Rb	Sr	Zn	Zn/Br	Zn/Fe	Zn/Rb	Zn/Sr
Age	-0.700[c]	-0.420[b]	0.022	0.168	0.292[a]	0.663[c]	0.600[c]	-0.002	-0.018

Statistically significant values: [a] $p \leq 0.05$, [b] $p \leq 0.01$, [c] $p \leq 0.001$.

variance accounted for (i.e. R^2) sense maximizes the value of R^2 using a linear, polynomial, exponential, logarithmic or power law for the approximation.

Discussion

The mean values and all selected statistical parameters were calculated for five TE (Br, Fe, Rb, Sr, and Zn) concentrations and for four Zn/TE (Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr) ratios in EPF samples (Table 2).

The mean of Zn concentration obtained for prostatic fluid, as shown in Table 3, agrees well with median of means cited by other researches.[15,19-26] The mean of Rb concentration obtained for EPF agrees well with our data reported 37 years ago.[22] No published data referring to Fe, Br, and Sr concentrations or Zn/TE ratios in EPF were found.

A statistically significant age-related decrease in Br concentration was observed in EPF when two age groups were compared (Table 5). In second group of males with mean age 59.1 years the mean of Br concentration in EPF was 2.2 times lower than in prostatic fluid of the first age group (mean age 27.5 years). A statistically significant decrease in Br concentration was confirmed by the negative Pearson's coefficient of correlation between age and concentration of this element (Table 5, Figure 1). In addition to this a significant decrease in Fe and increase in Zn concentration with increasing of age was shown by the Pearson's coefficient of correlation between age and concentration of the elements (Table 5, Figure 1). A change of Br concentration in the prostatic fluid with age from 18 to 82 years is more ideally fitted by a logarithmic law, Fe – by a linear law, and Zn by a polynomial law (Fig. 1). As per author's current information, no published data referring to age-related changes of TE concentration in human EPF is available with the exception of Zn.

Our finding for the Zn age-dependence does not agree with published data. For example, in the first quantitative X-ray fluorescent analysis of Zn concentration in prostatic fluid of 8 apparently healthy men aged 25-55 years no significant variation with age was recognized.[19] However, no any statistical treatment of results was done in this investigation. Using Atomic Absorption Spectrophotometry (AAS) for Zn measurement in prostatic fluid specimens obtained from 63 normal male subjects in age from 24 to 76 years Fair and Cordonnier[20] did not find any changes in metal level with age. The conclusion was followed from the level of differences between the mean Zn results for three age groups evaluated by parametric Student's *t*-test. Additionally, Zn, concentration in EPF showed no age relationship in the study of Kavanagh et al.[26] when 33 EPF specimens obtained from normal male subjects in age from 15 to 85 years were measured by AAS and the Pearson correlation between age and Zn concentration was used.

A statistically significant age-related increase in Zn/Br and Zn/Fe ratios was observed in EPF when two age groups were compared (Table 4). In addition to this a significant elevation of the Zn/Br and Zn/Fe ratios with increasing of age was shown by the Pearson's coefficient of correlation between age and Zn/TE ratios (Table 5, Figure 1). The changes of Zn/Br and Zn/Fe ratios in the EPF with age from 18 to 82 years were more ideally fitted by a polynomial law (Fig. 1). Because the Zn content on one hand and Br and Fe concentrations on the other one in EPF changed in opposite directions with increasing of age the changes of the Zn/Br and Zn/Fe ratios are more sensitive parameters than the absolute values of these TE. If the comparison of concentration of Br, Fe and Zn in EPS of two age group expressed difference as tens percentages, the values of Zn/Br and Zn/Fe ratios in normal EPF of males in the age range 41 to 82 years are almost 5.8 and 2.6 times, respectively, higher than those parameters in the age range 18 to 40 years. No published data on age-related changes of Zn/TE ratios in normal prostatic fluid were found.

Thus, if we accept the levels and relationships of TE concentrations in normal prostatic fluid of males in the age range 18 to 40 years as a norm, we must conclude that after age 40 years the level of Br, Fe and Zn concentrations as well as Zn/Br and Zn/Fe ratios in normal prostatic fluid significantly changed.

The range of means of Zn concentration reported in the literature for normal EPF (from 47.1 to 9870 mg/L) varies widely (Table 3). This can be explained by a dependence of Zn content on many factors, including age, ethnicity, mass of the gland, and others. Not all these factors were strictly controlled in cited studies. Another and, in our opinion, leading cause of inter-observer variability was insufficient quality control of results in these studies. Almost all analytical methods used for TE measurements in EPF were based on investigation of processed fluid with a goal to destroy and remove organic matrix. In such studies prostatic fluid samples were acid digested or dried under high temperature before analysis. There is evidence that by use of these methods some quantities of TE, including Zn, are lost as a result of this treatment.[28-30] Thus, when using destructive analytical methods it is necessary to control for the losses of TE, for complete acid digestion of the sample, and for the contaminations by TE during sample decomposition, which needs adding some chemicals. It is possible to avoid these not easy procedures using non-destructive methods, such as the ^{109}Cd radionuclide-induced EDXRF.

The ^{109}Cd radionuclide-induced EDXRF developed to determine TE concentrations in EPF is micro method because sample volume 20 µL (one drop) is quite enough for analysis. It is another advantage of the method. Amount of human EPF collected by massage of the normal prostate is usually in range 100-500 µL[27,31] but in a pathological state of gland, particularly after malignant transformation, this amount may be significantly lower. Therefore, the micro method of ^{109}Cd radionuclide-induced EDXRF developed to determine trace element concentrations in prostatic fluid is available for using in clinical studies.

Conclusion

The facility and method for ^{109}Cd radionuclide-induced EDXRF were developed to determine five TE (Br, Fe, Rb, Sr, and Zn) concentrations in the micro samples (20 µL) of EPF. The results of TE analysis in the micro samples are sufficiently representative for assessment of the Br, Fe, Rb, and Zn concentration in the prostatic fluid.

The means of Zn and Rb concentration obtained for EPF agree well with median of reported means. For the first time the Fe, Br, and Sr concentrations as well as Zn/Br, Zn/Fe, Zn/Rb, and Zn/Sr ratios were determined in the human EPS. Moreover an age-related increase in Zn and decrease in Br and Fe concentration accompanied by strongly pronounced increase in Zn/Br and Zn/Fe ratios was observed. Thus, the data does support our hypothesis about involvement of age-related changes of TE concentrations and their relationships in prostatic fluid in etiology and/or pathogenesis of prostate diseases and male infertility.

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Conflict of Interest.

The authors declare that they have no conflict of interest.

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