CORRELATION OF THE ZINC LEVEL IN THE SPERMOPLASM WITH THE FERTILITY CHARACTERISTICS OF HUMAN EJACULATE

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Background: *Zinc is essential for the normal functioning of the male reproductive system. The data on the diagnostic value of the zinc level in the human spermoplasm and its relationship with the main parameters of the sperm fertility are contradictory.* ***Aim:*** *study of the correlations between the zinc level in the spermoplasm and the spermogram characteristics.* ***Methods:*** *The sperm of men of the reproductive age (n=486, average age 33.07±3.03 years) was studied. In addition to the standard spermogram, MAR tests (IgA, IgG and IgM) were performed in the sperm samples, the degree of fragmentation of the sperm DNA, the sperm interaction with hyaluronic acid, the acrosine activity, and the neutral alpha-glucosidase activity were assessed, the citric acid, fructose and glycodelin levels were determined, and the level of reactive oxygen species was studied. The zinc level determination in the spermoplasm was carried out by a standard spectrophotometric method with 5-Br-PAPS chromogen. The Pearson's formula was used for the correlation analysis. The study was conducted from 2018 to May 2022, once.* ***Results:*** *A significant negative correlation of the zinc level in the spermoplasm with the age of men was revealed (r=-0.16; p <0.001). The level of zinc in the spermoplasm weakly negatively correlated with the dilution time and with the viscosity of the sperm. The positive correlation was found with the number of spermatozoa (r=0.13; p <0.01) and their mobility (r=0.38; p <0.00001). The level of zinc in the spermoplasm negatively correlated with the degree of the sperm DNA fragmentation and with the amount of reactive oxygen species, and positively correlated with the results of the test for binding to hyaluronic acid.* ***Conclusions:*** *The level of zinc in the spermoplasm significantly correlates with a number of physiological and biochemical characteristics of the sperm. The data obtained allow us to recommend determination of the zinc level in the sperm plasma to not only assess the functional activity of the prostate gland, but also to diagnose the fertility of the ejaculate, as well as to optimize the therapy with zinc-containing drugs and improve the control over the effectiveness of the treatment.*

Keywords: *zinc; ejaculate; sperm; fertility; male infertility.*

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BACKGROUND

Human sperm contains macro- and micro-elements that are crucial in the normal functioning of spermatozoa and their fertilizing properties [1–3]. Among these elements, zinc (Zn) is notable owing to its significantly higher concentration in sperm than in any other biological fluid in the human body [4].

Zinc is the only trace element included in the World Health Organization (WHO) recommendations for the study of human ejaculate as a biochemical marker of sperm fertility [5]. Zinc participates in the functioning of over three hundred metalloenzymes [6], making it an essential trace element [7].

Zinc metabolism disturbance can be caused by endogenous and exogenous factors, including natural geochemical anomalies in the distribution of this element found in several countries, such as Portugal, Iran, Egypt, Turkey, Panama, and some regions in Russia [8–10].

Zinc preparations have been used to treat male reproductive system diseases [6, 11]. However, the diagnostic value of determining this trace element in human sperm and its relationship with the main parameters of sperm fertility are contradictory, despite the critical role of zinc in the functioning of the male reproductive system [7, 11–13] (Table 1). Thus, for

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КОРРЕЛЯЦИЯ УРОВНЯ ЦИНКА В СПЕРМОПЛАЗМЕ С ХАРАКТЕРИСТИКАМИ ФЕРТИЛЬНОСТИ ЭЯКУЛЯТА ЧЕЛОВЕКА

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Обоснование. *Цинк имеет важное значение для нормального функционирования мужской репродуктивной системы. Данные о диагностическом значении определения цинка в спермоплазме человека и взаимосвязи его уровня с основными параметрами фертильности спермы носят противоречивый характер.* ***Цель исследования*** *— изучение корреляций уровня цинка в спермоплазме с характеристиками спермограммы.* ***Методы.*** *Исследована сперма мужчин репродуктивного возраста (n=486, средний возраст 33,07±3,03 года). В образцах спермы кроме стандартной спермограммы были выполнены MAR-тесты (IgA, IgG и IgM), проведена оценка степени фрагментации ДНК сперматозоидов и взаимодействия сперматозоидов с гиалуроновой кислотой; определены активность акрозина и нейтральной альфа-глюкозидазы, уровни лимонной кислоты, фруктозы и гликоделина; исследован уровень активных форм кислорода. Определение уровня цинка в спермоплазме проведено стандартным спектрофотометрическим методом с хромогеном 5-Br-PAPS. Для корреляционного анализа использована формула Пирсона. Исследование проводилось с 2018 г. по май 2022 г., однократно.* ***Результаты.*** *Выявлена достоверная отрицательная корреляция уровня цинка в спермоплазме с возрастом мужчин ($r=-0,16$; $p<0,001$). Уровень цинка в спермоплазме слабоотрицательно коррелировал со временем разжижения и вязкостью спермы. Положительная корреляция была с количеством сперматозоидов ($r=0,13$; $p<0,01$) и их подвижностью ($r=0,38$; $p<0,00001$). Уровень цинка в спермоплазме отрицательно коррелировал со степенью фрагментации ДНК сперматозоидов и количеством активных форм кислорода, а с тестом на связывание сперматозоидов с гиалуроновой кислотой — положительно.* ***Заключение.*** *Уровень цинка в спермоплазме достоверно коррелирует с рядом физиологических и биохимических характеристик спермы. Полученные данные позволяют рекомендовать определение цинка в спермоплазме не только для оценки функциональной активности предстательной железы, но и для диагностики фертильности эякулята, а также оптимизировать терапию цинксодержащими препаратами и улучшить контроль над эффективностью проводимого лечения.*

Ключевые слова: *цинк; эякулят; сперматозоиды; фертильность; мужское бесплодие.*

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example, in the study by Palani and Alshatteri [2], no correlation was found between zinc level in the sperm and sperm motility, morphology, and sperm count. However, other studies have reported a significant correlation between sperm plasma zinc level and sperm motility, morphology, and sperm count [16, 21]. Several studies have established a reliable correlation between sperm plasma zinc levels and sperm count [1, 19, 24] (Fig. 1). Furthermore, other studies have shown correlations between sperm plasma zinc levels and sperm morphology and motility, but not with sperm count [25], or only with motility [26] (Fig. 1b, c).

The inconsistent data (Fig. 1d) cannot be explained by the use of different methodological approaches to determine the level of zinc in sperm. This is particularly noteworthy because the main methods of determining the sperm plasma zinc level, namely, atomic absorption spectroscopy with flame atomization and direct

Table 1

KCorrelation of the zinc level in the spermoplasm with the main parameters of the spermogram according to the literature

Sperm count	Sperm morphology	Sperm motility	Total number of examined persons (fertile/subfertile)	Mean age of the examined persons (fertile/subfertile)	Method for zinc determination in sperm plasma	Year of publication	Literature
+	N	–	106 (8/98)	нд	ПК	1999	[14]
+	N	+	210 (103/107)	34.2±4.3/34.8±5.3	ААС-ПА	2000	[15]
+	N	N	210 (107/103)	нд	ААС-ПА	2001	[1]
+	+	+	170 (20/150)	нд	ААС-ПА	2005	[16]
+	+	N	72 (36/36)	30.44±3.83/30.02±3.88	ААС-ПА	2009	[17]
N	нд	N	99 (39/60)	31.87±3.76*	ПК	2009	[18]
+	N	N	152 (61/91)	33.43±5.10*	ААС-ПА	2010	[19]
+	N	+	1618 (318/1300)	33.09±0.59/34.33±0.37	ПК	2011	[4]
+	N	+	250 (60/190)	33.43±4.40/37.80±4.54	ААС-ПА	2012	[20]
+	+	+	52 (8/44)	нд	ААС-ПА	2013	[21]
нд	+	+	110 (50/60)	35.0±9.5*	ПК	2016	[22]
N	N	N	131 (25/106)	32.3±6.9/36.3±6.9	ОЭС-ИСП	2017	[2]
N	нд	N	106 (96/96)	30.2±5.3/31.2±5.9	ААС-ПА	2018	[3]
+	+	+	144	нд	ААС-ПА	2018	[23]
+	нд	нд	276 (176/100)	32 (30–37)/35 (31–38)	ПК	2020	[24]
N	+	+	70 (40/30)	36.80±4.91/37.74±5.41	ААС-ПА	2021	[25]
N	N	+	70	32.50±6.58*	ААС-ПА	2022	[26]

Note: * — the average age of all the examined persons; нд — the data are not provided by the author; N — no correlation found; «+» — reliable positive correlation; «–» — reliable negative correlation; ААС-ПА — atomic absorption spectroscopy with flame atomization; ПК — direct colorimetry (spectrophotometry); ОЭС-ИСП — optical emission spectrometry with inductively coupled plasma.

colorimetry (spectrophotometry) [27], showed a high degree of correlation (r=0.996; n=105). Further research is required to fully understand the relationship between zinc levels in sperm and fertility parameters, as evidenced by numerous research studies, including reviews and meta-analyses.

This study aimed to investigate the correlation between sperm plasma zinc levels and spermogram characteristics.

METHODS

Study design

Multicenter observational cohort retrospective study.

Eligibility criteria

Inclusion criteria: male gender.

Noninclusion criteria: prostate cancer, Danbolt–Closs syndrome, Prasad's disease, bad habits (smoking and alcoholism), treatment with zinc-containing drugs, and drugs that may have a negative effect on male fertility [28].

Exclusion criteria: azoospermia.

Settings

This study was conducted at the DIAMED-Express Laboratory of REPRODIAMED LLC and Clinical Diagnostic Laboratory of the Alexandro-Mariinsky Regional Clinical Hospital in Astrakhan, Russia.

Duration of the study

The study was conducted between 2018 and May 2022.

Description of the medical intervention

To achieve this goal, patients who requested spermograms underwent a comprehensive analysis of the ejaculate and determination of sperm zinc level.

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In addition to the standard spermogram following the WHO protocol [5], the comprehensive semen analysis included a mixed aggregation reaction (MAR) test or direct immunobead test to detect antisperm antibodies (immunoglobulins of isotypes A, G, and M). The following tests were conducted on the sperm surface: determination of citric acid, fructose, neutral α-glucosidase, and acrosin activity in sperm plasma. Additionally, the degree of sperm DNA fragmentation (SDF test), sperm hyaluronic acid interaction, and oxidative stress level were evaluated.

To assess the results of the spermogram, normative values recommended by the WHO expert group [5] and generally accepted in laboratory diagnostics when examining the ejaculate [29] were used (Table 2).

The MAR test was performed using commercial kits SpermMar IgA and SpermMar IgG (FertiPro, Belgium) and ImmunoSpheres Anti-IgM (BioScreen Inc., USA). This study was conducted in accordance with the recommendations of the WHO expert group [29].

Citric acid and fructose levels in sperm plasma were determined using spectrophotometric methods and commercial test kits (FertiPro, Belgium). The optical density was measured at wavelengths of 405 and 492 nm, respectively [29].

The spectrophotometric method was used to determine the activity of neutral α-glucosidase, with p-nitrophenyl-α-D-glucopyranoside as the substrate. A commercial EpiScreen Plus kit (FertiPro, Belgium) was utilized. The optical density was measured at a wavelength of 405 nm [29].

To determine the degree of SDF, we used the Sperm Chromatin Dispersion (SCD) method, which assesses the susceptibility of sperm DNA to acid denaturation. After denaturation and nuclear protein extraction, intact DNA strands are exposed, whereas DNA fragmentation results in no or minimal dispersion. The SCD method relies on the ability of intact sperm chromatin to form halos of dispersion (halo) after exposure to acid and leading solution. The halos correspond to unfolded DNA loops that are attached to the residual structures of the nucleus that form after nuclear protein removal. Halo formation is prevented by DNA breaks that are subject to denaturation. The formed halos were detected using light microscopy [29]. Commercial kits GoldCyto DNA (Guangzhou Jinsaite Trading, China) and Halosperm G2 (Halotech DNA, Spain) were used.

The hyaluronan binding assay (HBA) test was conducted on slides containing immobilized hyaluronic acid using a commercial HBA Assay kit (Biocoat Inc., USA) [29].

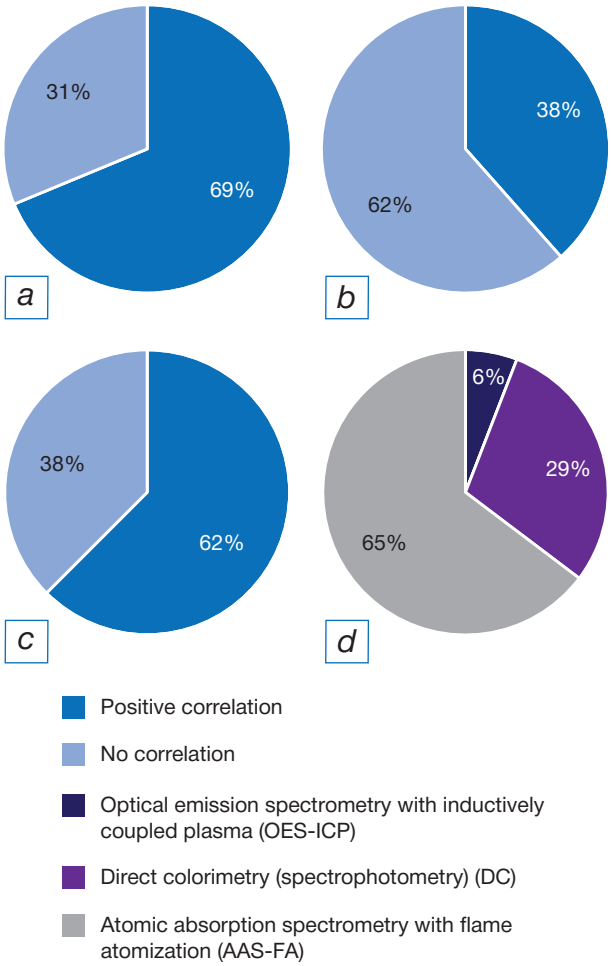

Fig. 1. Correlation of zinc levels with sperm parameters according to the literature. Frequency of zinc relationship with the amount (*a*); morphology (*b*) and motility (*c*) of spermatozoa; (*d*) — methods for determining zinc in spermoplasm.

Acrosin activity was determined using a standard spectrophotometric method with N-α-benzoyl-DL-arginine-p-nitroanidide as a substrate and a commercial AcroScreen kit (Bioscreen Inc., USA). The optical density was measured at a wavelength of 405 nm [29].

Glycodelin concentration in sperm plasma was measured using the AMGF Fertitest-M commercial kit (Diatex-EM LLC, Russia) through solid-phase enzyme immunoassay in sandwich modification.

Reactive oxygen species (ROS) were detected using a colorimetric method with nitroblue tetrazolium (NBT). This method is based on the ability of the superoxide anion radical to reduce water-soluble NBT to water-insoluble formazan, resulting in blue formazan crystal formation [29]. The commercial OxiSperm kit (Halotech DNA, Spain) was used in this study.

Table 2

Reference values of the ejaculate fertility indicators

Characteristics of the ejaculate	Spermogram parameters	Units of measurement
Volume	>1.5	mL
Ejaculate viscosity	<2.0	cm
Total sperm count	>40.0	$\times 10^6$
Sperm concentration	>15.0	$\times 10^6$/mL
Actively motile spermatozoa ("*a*" category)	>25.0	%
Progressively motile spermatozoa ("*a*+*b*" category)	>50.0	%
Sperm viability (vital staining and/or HOS test)	>58.0	%
Normal sperm shapes	>4.0	%
pH	≥7.2	-
Leukocytes	<1.0	$\times 10^6$/mL
Nonspecific sperm aggregation	Absent	-
Sperm agglutination	Absent	-
MAR test (or IBD test)	<50.0	%
HBA test	>80.0	%
SDF test	<15.0	%
Zinc	>150.0	mcg/ejaculate
Citric acid	>10.0	mg/ejaculate
Fructose	>2.4	mg/ejaculate
Neutral alpha-glucosidase	>20.0	mME/ejaculate
Acrosin	50.0 to 250.0	μME/ 10^6 spermatozoa
Glycodelin-S	20.0 to 200.0	mcg/mL
Reactive oxygen species	Level 1 (low)	-

Zinc levels in sperm plasma were determined by the standard spectrophotometric method [29] in which the chromogen 5-Br-PAPS [2-(5-nitro-2-pyridylazo)-5-(N-propyl-N-sulfopropylamino)-phenol] binds to zinc and changes color:

$$5\text{-Br-PAPS} + Zn^{2+} \rightarrow 5\text{-Br-PAPS-Zn}.$$

The 5-Br-PAPS-Zn complex absorbs light at a wavelength of 560 nm (550–580). The optical density of the resulting stable complex is proportional to the zinc content of the sample.

The Zinc Sp-DAC.Lq commercial kit (DAC-SpectroMed s.r.l., Republic of Moldova) was used to determine the optical density at 570 nm. The linearity limits were 0–10.0 μg/mL (equivalent to 1000.0 μg/mL in the whole sample). The intra-series coefficient of variation (reproducibility within the period) was CV ≤3.0%. The inter-series coefficient of variation (reproducibility from period to period) was CV ≤6.5%.

Ethical review

This study was conducted in accordance with the ethical standards of the World Medical Association Declaration of Helsinki "Ethical Principles for Scientific Medical Research Involving Human Subjects," as amended in 2013.

Statistical analysis

For statistical analysis, Student's t-test and the linear correlation coefficient (Pearson's formula) with the MedCalc Ver.19.8 software package (MedCalc Software Ltd., Belgium) were used. p <0.05 indicated statistical significance.

RESULTS

Objects (participants) of the study

In total, 510 patients who met the inclusion and noninclusion criteria were examined. Of these, 24 (4.7%) were diagnosed with azoospermia, and, in accordance with the stated criteria, their ejaculates were excluded from further analysis.

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The remaining 486 men, aged 17–68 years, participated in the study. This group included 407 subfertile patients and 79 conditionally healthy patients with confirmed fertility (children under one and a half years of age). Table 3 presents a more detailed characterization.

Main results of the study

A significant negative correlation was found between the age of men and zinc concentration in sperm (p <0.001). On average, the level of zinc in sperm was significantly lower in men aged <30 years than in men aged ≥40 years (Fig. 2). Despite significant individual differences, this trend was observed.

Analysis of the physicochemical properties of ejaculate compared with sperm plasma zinc levels revealed weak negative correlations with liquefaction time and semen viscosity (r=-0.0984 and r=-0.0917, respectively; p <0.05) (Table 4).

Regarding the primary characteristics of sperm fertility, the level of zinc in sperm plasma exhibited a weak but significant correlation (r=0.1345, p <0.01) with the number of spermatozoa in the ejaculate. The correlation with sperm motility was stronger (r=0.3825; p <0.00001) (Table 4).

Sperm morphology is a crucial factor in determining fertility, along with sperm count and motility. However,

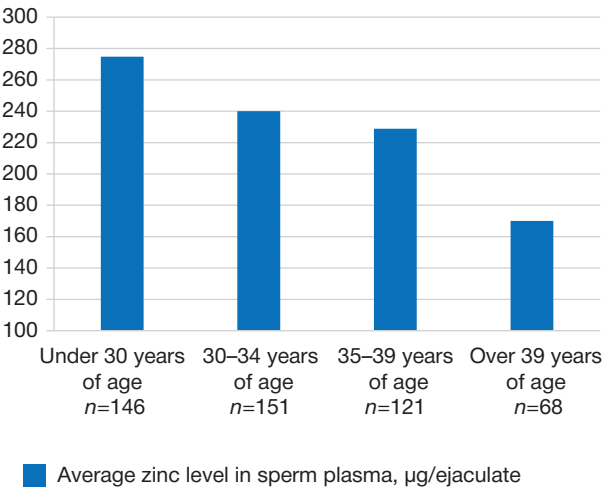

Fig. 2. The average level of zinc in the spermoplasm in men of different age groups.

Table 3

Characteristics of the examined group of patients

Characteristics	All examined	Fertile	Subfertile
Number, *n*	486	79	407
Average age, years	33.07±3.03	33.12±3.06	33.06±3.02
Minimum age, years	17	20	17
Maximum age, years	68	68	58

Table 4

Correlation between the zinc level in the spermoplasm and the spermogram parameters

Correlation of zinc level in sperm plasma (μg/ejaculate), *n*=486	Linear correlation coefficient (Pearson formula), *r*	*p*
Patient's age	-0.1625	<0.001
Sperm count	0.1345	<0.01
Sperm motility	0.3825	<0.00001
Sperm morphology	Not correlated	Unreliable
Ejaculate viscosity	-0.0917	<0.05
Ejaculate liquefaction time	-0.0984	<0.05
ejaculate pH	Not correlated	Unreliable
Sperm viability	0.1381	<0.01
Concentration of leukocytes in the ejaculate	-0.1180	<0.01
MAR test (IgA)	Not correlated	Unreliable
MAR test (IgG)	Not correlated	Unreliable
MAR test (IgM)	Not correlated	Unreliable
SDF test	-0.1907	<0.0001
HBA test	0.1201	<0.01

analysis of zinc levels in the sperm of ejaculates with varying proportions of normal and defective spermatozoa did not reveal a significant correlation.

Zinc levels in sperm showed a weak positive correlation with sperm viability (Table 4).

Leukocytes are typically present in semen; however, a concentration >10^6/mL is considered leukospermia and may indicate an inflammatory process, often of infectious nature, in the male reproductive system. Our study found a significant weak negative correlation (r=-0.1180; p <0.05) between leukocyte and zinc levels.

No significant correlation was observed between the presence of antisperm antibodies of different classes (IgA, IgG, and IgM) on the spermatozoa (MAR test) and the level of zinc in sperm plasma.

Investigation beyond the standard spermogram showed that the degree of sperm DNA integrity disruption (detected by the SDF test) was negatively correlated with sperm plasma zinc levels (Table 4).

The HBA assesses the fertilizing capacity of sperm by evaluating their interaction with the zona pellucida, the transparent membrane of the oocyte. A weak positive correlation was found between the HBA results and zinc levels in sperm (r=0.1201; p <0.01)

This study analyzed the correlations between biochemical parameters of ejaculate and zinc levels. The results showed reliable positive correlations between "zinc–citric acid" (p <0.001) and "zinc–acrosin activity" (p <0.00001) and a weak negative correlation in "zinc–ROS" (p <0.01) (Table 5).

However, zinc levels in sperm did not correlate with fructose concentration, glycodelin level, or neutral alpha-glucosidase activity.

DISCUSSION

This study confirms earlier findings of a significant negative correlation between zinc level in ejaculate and the age of men [9]. However, some studies have not observed a relationship between zinc level in sperm and age. For example, Bazid et al. [26] have found no reliable correlation between zinc levels in semen and age, which we believe is because of the small sample size (n=70). The correlation coefficient obtained was low (r=-0.0984; p <0.001; n=486); however, it was characterized by sufficient reliability, confirming that the average zinc level in sperm plasma decreases with age (Fig. 2).

Studies on the correlation between sperm plasma zinc levels, liquefaction time, and semen viscosity are limited. Our study revealed nonsignificant but noteworthy (p <0.05) negative correlations between sperm plasma zinc levels and ejaculate liquefaction time and semen viscosity, which is consistent with previous research [19]. The extended time required for ejaculate liquefaction, which is associated with reduced zinc levels, may be due to a decrease in the activity of zinc-dependent metalloenzymes involved in liquefying the sperm that has coagulated after ejaculation.

Examination of 486 samples did not confirm the correlation between zinc levels in sperm plasma and ejaculate pH, as previously stated by Dissanayake et al. [19] (r=-0.193; p <0.05; n=152).

Most studies focused on the relationship between the zinc level in sperm plasma and the most crucial characteristics of ejaculate fertility, namely, the number, motility, and morphology of spermatozoa. The most contradictory results are associated with these parameters. The contradictions are related to insufficient representativeness: in many papers, the number of observations does not exceed 200 semen samples (Table 1), and sometimes even less than 100 (n=99 [18], n=72 [17], n=70 [25], n=70 [26], and n=52 [21]). In these five studies, only 363 ejaculate samples were investigated, and conflicting data were obtained. The sample size seems insufficient to detect weak correlations and causes confusion when interpreting the results obtained.

Table 5

Correlation between the level of zinc and other biochemical components of the spermoplasm

Correlation of zinc level in sperm plasma (µg/ejaculate), n=486	Linear correlation coefficient (Pearson formula), r	p
Concentration of citric acid	0.1541	<0.001
Fructose concentration	Not correlated	Unreliable
Glycodelin-S concentration	Not correlated	Unreliable
Neutral alpha-glucosidase activity	Not correlated	Unreliable
Acrosin activity	0.2137	<0.00001
Reactive oxygen species level	-0.1212	<0.01

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Our data (*n*=486) showed a weak positive correlation between zinc level in sperm plasma and the number of spermatozoa (p <0.01). This correlation may be due to the role of zinc in spermatogenesis during mitosis of spermatogonia and meiosis of spermatocytes, such as regulating key enzymes including ribonuclease [7, 30]. Our data support that of a previous study (*n*=1618) that found a 20% lower average level of zinc in the sperm plasma of patients with oligozoospermia than in that of patients with normozoospermia [4].

A meta-analysis of the relationship between sperm plasma zinc levels and sperm motility characteristics revealed that sperm plasma zinc levels are lower in ejaculates with asthenozoospermia than in those with normal sperm motility [31]. Testicular and epididymal spermatozoa contain less intracellular zinc than ejaculated spermatozoa (2.56±0.51 and 12.58±3.16 vs. 40.48±12.71 ng Zn per 106 spermatozoa, respectively) [32]. Zinc is present in the mitochondria of spermatozoa and along the flagella [12]. The balance of zinc within and outside cells is maintained through a system of receptors and transporter proteins. One of the pathways that regulate transport involves the following cascade: GPR39 (a zinc-sensing receptor of the G protein-coupled receptor 39 type) — adenylyl cyclase — cyclic adenosine monophosphate (cAMP) — protein kinase A — SRC tyrosine kinase — epidermal growth factor receptor — phospholipase C [33]. The transmission of signals of secondary messengers (cAMP) is organized in subcellular domains (head, main part of flagellum, end part of flagellum) in spermatozoa [34]. This organization allows for sperm motility regulation, including timely stimulation of hyperactivity by maintaining an optimal zinc balance within the spermatozoa [35]. This balance requires a sufficient level of zinc in the sperm. A significant correlation was observed between zinc level and sperm motility (p <0.00001). The correlation between zinc levels in sperm and acrosin activity is explained by similar regulatory mechanisms. This is because the GPR39 receptor is found in the flagellum and acrosome of spermatozoa [33], and zinc can stimulate acrosomal exocytosis in mammalian spermatozoa [12, 36].

In contrast to other studies, the present study did not find a significant correlation between sperm plasma zinc levels and sperm morphology. The correlations found by other researchers were possibly due to insufficient sample size (e.g., *n*=70 [25]; *n*=52 [21]) or the nonrandom nature of the sample (e.g., the presence of urogenital chlamydia) [22].

The correlation between zinc levels in sperm plasma and sperm viability may be due to the trace element in stabilizing sperm membranes. Zinc regulates the phase state of membrane lipids [7, 15], participates in antioxidant protection [6, 37], and may play a role in regulating sperm ferroptosis [38].

A previous study has revealed a significant correlation (r=0.40; p <0.05; *n*=144) between zinc level in sperm and antioxidant activity of sperm [23]. Moreover, a significant (p <0.01) negative correlation was observed between zinc level in sperm and level of ROS in the ejaculate. Zinc participates in the antioxidant defense system through zinc-containing enzymes, such as superoxide dismutase, and nonenzymatic mechanisms [7].

The correlation between zinc level in sperm plasma and leukocyte concentration in the ejaculate is weakly negative, as confirmed by our observations and previous data [39]. A decrease in zinc level in semen can indicate impaired functional activity of the prostate gland in prostatitis, which often causes leukospermia [40, 41]. Urogenital infections are the most common cause of leukospermia. In some cases, these infections may decrease zinc levels in sperm plasma. However, this is not always the case. For instance, Ziganshin et al. [22] have found a significant reduction in sperm plasma zinc level in cases of urogenital chlamydia. In contrast, our data showed no significant change in the level of zinc in sperm in cases of urogenital ureaplasmosis [42]. Systemic inflammatory processes can affect sperm plasma zinc levels. Some studies have shown a decrease in sperm plasma zinc levels (p ≤0.05; *n*=17) after COVID-19, along with SDF ≥15% [43]. However, in our study (*n*=144) of asymptomatic and mild COVID-19 cases, any significant changes were not noted in sperm plasma zinc levels [44]. The contradictions mentioned above can be explained by the impact of different pathogens on the macroorganism and the varying severity of the infectious process.

The negative correlation between sperm plasma zinc level and the degree of SDF is consistent with previously obtained data [45]. Zinc participates in the formation of S–Zn–S bonds in the protamine structure, acts as a regulator of disulfide cross-links in the sperm nucleus [7], and improves DNA methylation and chromatin integrity under toxic effects [46].

The correlation between sperm zinc level and the results of the HBA test may be due to the ability of zinc to regulate receptor affinity through conformational changes. This modulation affects the interaction between sperm and the *zona pellucida* [47, 48].

The correlation between zinc and citric acid levels in sperm is positive because of their common biosynthesis site, the prostate gland [40]. Furthermore, zinc secretion occurs partially in complex with citric acid, which acts as a ligand [7].

In contrast to some studies [49], this study did not observe any correlation between sperm zinc level and neutral alpha-glucosidase (which originates from the epididymis) activity or fructose and glycodelin (which are secreted by the seminal vesicles) levels in sperm.

CONCLUSIONS

Thus, sperm plasma zinc level correlates with various physiological and biochemical characteristics of semen, indicating that sperm plasma zinc levels can be measured to evaluate the functional activity of the prostate gland, diagnose ejaculate fertility, optimize therapy with zinc-containing drugs, and improve the monitoring of treatment effectiveness.

ADDITIONAL INFORMATION

Authors' contribution. *D.L. Lutsky* — research concept and design, statistical data analysis, manuscript writing; *R.M. Makhmudov* — statistical data analysis; *A.M. Lutskaya* — collection and processing of material, manuscript writing; *E.V. Palkina, A.I. Polunin* — collection and processing of the date, manuscript editing. The authors made a substantial contribution to the conception of the work, acquisition, analysis, interpretation of data for the work, drafting and revising the work, final approval of the version to be published and agree to be accountable for all aspects of the work.

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