REVIEW

Seminal plasma metallomics: a new horizon for diagnosing and managing male infertility

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Abstract

Seminal plasma contains a wide range of biomolecules—including inorganic elements—that may significantly influence male reproductive function. Historically, semen analysis has focused on sperm count and motility, while overlooking the diagnostic potential of this acellular fraction. This narrative review synthesizes historical perspectives on seminal plasma metallomics, elucidates the biological functions of its diverse elemental constituents, and critically evaluates methodological advancements in their detection. Furthermore, it examines future clinical and research directions by addressing key topics, including the evolution of multi-element analyses in seminal plasma, the interplay between metal exposure and male reproductive health, and the application of omics-based and machine-learning approaches in characterizing male infertility. Progress in analytical chemistry, particularly inductively coupled plasma mass spectrometry, now enables high-precision multi-element measurements in seminal plasma. The "metallomic" profiles reveal both essential elements—such as calcium, magnesium, potassium, sodium, zinc and selenium—and potentially toxic metals, including cadmium and lead, that reflect environmental exposures and may impair fertility. Seminal plasma metallomics also underscores fraction-specific differences between prostatic and seminal vesicular secretions, suggesting that certain chemicals may rise in seminal plasma before shifts appear in blood, thereby making it a promising biomarker for infertility risk assessment. Machine-learning approaches, such as clustering based on seminal plasma-to-serum ratios, offer new diagnostic insights by identifying subtypes of male infertility. By complementing traditional semen parameters and advanced biomarkers (e.g., DNA fragmentation index), these integrative tools can refine diagnoses and guide interventions, including nutritional supplementation and avoidance of specific toxicants, potentially improving pregnancy outcomes. However, significant challenges remain: standardized protocols, validated reference ranges, and larger prospective studies are needed for clinical translation. Addressing these gaps is crucial for integrating metallomic analyses into routine evaluations of male infertility. As this field continues to evolve, it has the potential to reshape infertility assessments and foster more personalized and effective management strategies.

Keywords

Male infertility; Seminal plasma; Metallomics; Trace elements; Environmental exposure; Machine learning; Personalized medicine; Semen analysis; Zinc; Phosphorus

1. Introduction

Infertility in humans often occurs when an insufficient number of spermatozoa reaches the female oviduct following vaginal coitus or intrauterine insemination (IUI), thereby preventing fertilisation. Historically, semen analysis has primarily emphasised sperm count and motility to diagnose male infertility. However, seminal plasma (i.e., semen without cells) contains a myriad of lipids, inorganic ions, metabolites, nucleic acids, proteins and other biomolecules, the physiological roles of which remain underexplored [1–4]. In addition to its contribution to fertilisation, seminal plasma has gained attention as a potential reservoir of biomarkers relevant not only to fertility but also to genitourinary malignancies and infections [5,6].

Metallomics is a branch of analytical chemistry that systematically investigates metals and metalloids in biological systems [7]. Seminal plasma metallomics specifically aims to characterise the inorganic constituents (e.g., metal ions and metal–protein complexes) in semen. In this review, we use the term “metals” broadly to encompass all inorganic elements relevant to metallomics, including certain metalloids and, on rare occasions, non-metals (e.g., phosphorus). More than a century has passed since the initial suggestion that zinc is crucial for vertebrate reproduction. Recent omics-based approaches have widened the scope of the metals/metalloids under investigation. These developments have potentially transformed diagnostics in reproductive medicine by highlighting trace elements in seminal plasma that can affect sperm physiology or reflect environmental exposure.

Building on previous studies that explored the clustering of male infertility subtypes using seminal plasma-to-serum trace element concentration ratios [8], we undertook this narrative review as part of a broader cross-sectional project aimed at developing new diagnostic strategies for male infertility. To identify relevant literature, we performed a focused search of PubMed and Google Scholar using terms such as “male infertility”, “seminal plasma”, “trace elements”, “metallomics” and “environmental exposure”. We also examined several specialized andrology textbooks to confirm certain historical milestones and methodological details. By

incorporating these references into our broader search strategy, we aimed to capture both the foundational and the most current perspectives on seminal plasma metallomics in male infertility. Through this process, we synthesized historical perspectives on the field, examined the biological functions of various elemental constituents and evaluated methodological advancements in their detection. In the following sections, we address key topics such as the evolution of multi-element analyses in seminal plasma, the interplay between metal exposure and male reproductive health, and the potential of metallomics-based approaches to refine the classification and management of male infertility.

2. Historical perspectives on seminal plasma trace element research

2.1 Early observations: zinc and fertility (1920s–1970s)

Initial hints of the relevance of trace elements in semen date back to the 1920s, when Bertrand and Vladesco proposed that zinc plays a role in vertebrate reproduction [9]. Research in subsequent decades has confirmed that zinc and other ions are present in male accessory gland secretions and influence sperm function. However, until the 1970s, analytical limitations restricted most investigations to measuring a small number of elements—primarily zinc, calcium and magnesium—in relation to spermatozoal parameters [10].

2.2 Emergence of multi-element analytical techniques (1980s–2000s)

In the 1980s, atomic absorption spectrometry and early inductively coupled plasma mass spectrometry (ICP-MS) expanded the range of elements detectable in semen [11]. Studies have begun to compare fertile and infertile men, with a focus on the detection of single heavy metals, such as lead or cadmium, along with essential elements. Despite technological advances, many surveys lack precise information regarding the exposure history or environmental confounders [12].

2.3 Rise of metallomics and modern seminal plasma studies

After 2010, advances in ICP-MS enabled simultaneous measurement of multiple ultra-trace elements, spurring integrative analyses of seminal plasma "metallomes" [13]. Researchers have increasingly recognised that many elements beyond zinc can influence sperm function, either as essential micronutrients or as

toxicants. New high-throughput approaches allow investigators to examine previously unstudied metals in normal and abnormal semen, thereby offering a broader perspective on male reproductive function.

3. Biological significance of trace elements in seminal plasma

3.1 Intracellular vs. extracellular elemental concentrations

Body fluids generally comprise cellular components and a fluid fraction. In blood, erythrocytes constitute about 40–45% of the total volume (roughly 4–6 × 10^9 erythrocytes per mL), whereas the fluid component is plasma; in clinical practice, serum is derived by allowing blood to clot, thereby removing clotting factors (particularly fibrinogen) from the fluid component. Many electrolytes and trace elements—such as sodium, potassium, calcium, magnesium and zinc—are routinely measured in serum, reflecting extracellular concentrations. However, certain elements (e.g., cadmium and lead) can accumulate within erythrocytes over their 120-day lifespan, making whole-blood analysis more appropriate for assessing chronic exposure in occupational or environmental contexts [14]. The standard concentrations of representative elements in whole blood, erythrocytes and serum are summarized in Table 1 (Ref. [14–16]).

Table 1. Elemental concentrations in whole blood, serum and erythrocytes.

Z	Element	Classification	Concentration (mg/L)		
			Whole blood	Serum	Erythrocyte
11	Sodium	Light metal	1.7×10^3–2.0×10^3 [a]	3.0×10^3–3.4×10^3 [a]	1.8×10^2–3.6×10^2 [b]
12	Magnesium	Light metal	3.0×10^1–3.9×10^1 [a]	1.9×10^1–2.4×10^1 [a]	4.6×10^1–6.3×10^1 [a]
15	Phosphorus [c]	Non-metal / Metalloid	3.2×10^2–3.9×10^2 [a]	1.2×10^2–1.8×10^2 [a]	5.6×10^2–7.6×10^2 [a]
19	Potassium	Light metal	1.5×10^3–1.8×10^3 [a]	1.5×10^2–2.3×10^2 [a]	3.0×10^3–4.0×10^3 [a]
20	Calcium	Light metal	4.8×10^1–6.0×10^1 [a]	8.8×10^1–1.0×10^2 [a]	6.3×10^{-1}–5.2×10^0 [b]

Z	Element	Classification	Concentration (mg/L)		
			Whole blood	Serum	Erythrocyte
25	Manganese	Heavy metal	5.0×10^{-3}–1.4×10^{-2} [a]	4.0×10^{-4}–6.0×10^{-4} [a]	8.9×10^{-3}–2.9×10^{-2} [a]
26	Iron	Heavy metal	4.0×10^{2}–5.1×10^{2} [a]	8.7×10^{-1}–1.9×10^{0} [b]	9.6×10^{2}–1.2×10^{3} [a]
29	Copper	Heavy metal	7.3×10^{-1}–1.4×10^{0} [a]	8.0×10^{-1}–1.9×10^{0} [a]	5.4×10^{-1}–7.5×10^{-1} [a]
30	Zinc	Heavy metal	4.7×10^{0}–6.7×10^{0} [a]	7.3×10^{-1}–1.1×10^{0} [a]	1.0×10^{1}–1.5×10^{1} [a]
33	Arsenic	Metalloid	7.0×10^{-5}–3.4×10^{-3} [a]	3.0×10^{-5}–1.7×10^{-3} [a]	1.6×10^{-4}–5.8×10^{-3} [a]
34	Selenium	Metalloid	8.5×10^{-2}–1.3×10^{-1} [a]	7.0×10^{-2}–1.1×10^{-1} [a]	1.1×10^{-1}–1.9×10^{-1} [a]
48	Cadmium	Heavy metal	1.3×10^{-4}–1.7×10^{-3} [a]	$<9.0\times10^{-6}$–1.7×10^{-5} [a]	2.1×10^{-4}–3.4×10^{-3} [a]
82	Lead	Heavy metal	5.4×10^{-3}–2.6×10^{-2} [a]	1.0×10^{-5}–1.0×10^{-4} [a]	1.2×10^{-2}–6.3×10^{-2} [a]

Z Atomic number.

[a] Values quoted from Heitland and Köster (2021) [14], representing the 5th–95th percentile range (approximate values rounded to two significant figures).

[b] Derived from the review by Iyengar et al. [15] (1978), showing the minimum and maximum among reported mean concentrations in the literature examined (approximate values rounded to two significant figures).

[c] Phosphorus is widely recognized as a non-metal, although certain sources have classified it as a metalloid. The values here represent total phosphorus, encompassing not only inorganic phosphate but also phospholipids and other phosphorus-containing compounds, and thus differ from the inorganic phosphate typically measured in routine clinical practice [16].

A similar logic applies to semen: it has a cellular component (sperm) and a fluid component (seminal plasma). Yet sperm concentrations (up to~1×10^{8} per mL) are generally lower than erythrocytes counts in blood, meaning the impact of "cell removal" (i.e., separating sperm) on trace element measurements can be less dramatic than that between whole blood and serum. Nevertheless, studies often

prefer to measure seminal plasma specifically, in order to focus on the microenvironment that directly surrounds sperm.

Unlike serum, methodological standardisation for electrolytes and trace elements in seminal plasma remains limited. Reported concentrations vary widely across investigations, potentially reflecting genuine differences in population, region, diet and environmental exposure, but also inconsistencies in sample processing or analytical protocols. Consequently, caution is warranted when comparing absolute values between studies. As an alternative, we and others have proposed ratio-based approaches (e.g., "seminal plasma-to-serum ratios") to help reduce inter-laboratory variability [8]. While some groups use serum and seminal plasma in parallel [17], other group simultaneously evaluated "whole blood" and "whole semen," underscoring the importance of clarifying which compartments are being measured in reproductive toxicology research [18]. Table 2 (Ref. [8,16,19–22]) provides a concise overview of key trace elements in seminal plasma—including their typical distribution, primary glandular sources and reproductive roles—which will be referenced throughout this manuscript where relevant.

Table 2. Seminal plasma trace elements: distribution, origins and roles.

Z	Element	SP/Se ratio [a]	Dominant glandular origin	Potential role in seminal plasma [12,19,20]
11	Sodium	0.88–0.97 [b]	Prostate [c]	Maintains osmotic balance and membrane potential essential for sperm viability
12	Magnesium	2.9–7.3 [b]	Prostate [c]	Supports ejaculatory function and stabilizes sperm membranes
15	Phosphorus [d]	6.12–9.24 [b]	Seminal vesicle [c]	Contributes to energy metabolism and acid phosphatase activity, aiding sperm function
19	Potassium	5.37–9.00 [b]	Prostate [c]	Maintains osmotic balance and membrane potential essential for sperm viability
20	Calcium	2.18–4.55 [b]	Prostate [c]	Regulates sperm motility and acrosome reaction
25	Manganese	7–18 [b]	Prostate [c]	Acts as a cofactor in antioxidant defense; exact role in sperm function under investigation
26	Iron	0.08–0.16 [b]	Prostate [c]	No major direct role; may contribute to oxidative balance in seminal plasma

Z	Element	SP/Se ratio [a]	Dominant glandular origin	Potential role in seminal plasma [12,19,20]
29	Copper	0.08–0.16 [b]	Prostate [c]	Acts as a cofactor for antioxidant enzymes; may indirectly influence sperm quality
30	Zinc	116–306 [b]	Prostate [c]	Stabilizes sperm chromatin and supports antioxidant defense
33	Arsenic	1.61–3.49 [b]	Seminal vesicle [c]	Non-essential; can disrupt sperm parameters when elevated
34	Selenium	0.37–0.61 [b]	Prostate [c]	Essential for selenoproteins; protects sperm from oxidative stress
48	Cadmium	Unknown [e]	Unknown [f]	Non-essential; accumulates in tissues and may impair testicular function
82	Lead	Unknown [e]	Seminal vesicle? [f]	Non-essential; interferes with reproductive hormones and sperm parameters

Z Atomic number.

SP Seminal plasma.

Se Serum.

[a] The value indicates the relative concentration in seminal plasma when the serum concentration is set to 1.

[b] These data refer to our findings in Tanaka (2024) [8]. Specifically, they represent the 25th–75th percentiles for men whose partners conceived within one year without undergoing in vitro fertilization or intracytoplasmic sperm injection.

[c] Based on our own data in Tanaka (2024) [8] using split ejaculate sampling: elements showing higher concentrations in the early fraction were deemed prostate-dominant, whereas those showing higher concentrations in the later fraction were deemed seminal vesicle–dominant.

[d] Here, the phosphorus values represent total phosphorus, encompassing not only inorganic phosphate but also phospholipids and other phosphorus-containing compounds, and thus differ from the inorganic phosphate typically measured in routine clinical practice [16].

[e] No systematic study has established SP/Se ratios for cadmium or lead. Althogh the data from Riaz (2016) [21] may be partially informative, they may overestimate serum concentrations compared with other studies.

[f] Based on Pant (2003) [22], cadmium's lack of correlation with fructose or acid phosphatase leaves its origin unclear, whereas lead's positive correlation with fructose and negative correlation with acid phosphatase suggests the seminal vesicles as its likely dominant origin.

3.2 Essential elements

Many essential elements are found in their ionic forms in seminal plasma and support sperm physiology [20] (Table 2). Calcium modulates sperm motility, hyperactivation, acrosome reaction and chemotaxis [23]. Magnesium is critical for the ejaculatory function and affects sperm membrane stability [24,25]. Potassium and sodium are vital for membrane potential regulation via sodium–potassium adenosine triphosphatase (Na/K-ATPase) [26]. Zinc helps stabilise sperm chromatin, assists in antioxidant defense and supports spermatogenesis [27,28]. Selenium is a cofactor in selenoproteins that protect cells from oxidative damage and is integral to sperm formation [29].

These elements often differ in absolute concentration between blood and seminal plasma. For example, prostatic fluid typically contains high levels of zinc, reflecting a specialized role in stabilizing sperm DNA and supporting accessory gland function. Assessing such elements in the reproductive tract's fluid fraction can thus provide information beyond conventional serum measurements alone.

3.3 Potentially toxic metals and metalloids

Heavy metals, defined as inorganic elements with a density greater than 5 g/cm^3 [30], are often associated with toxicity when present in excess (Tables 1 and 2). Examples include silver, arsenic, cadmium, chromium, cobalt, copper, lead, mercury, nickel and zinc. These metals are classified as non-essential or in cases where they may be biologically essential, can become harmful when their levels exceed the physiological requirements [31]. Certain heavy metals tend to accumulate in biological materials owing to the limited detoxification and excretion pathways in the body, making them reliable environmental exposure biomarkers for assessing health risks [32].

The reproductive system is particularly vulnerable to the adverse effects of heavy metals, which act through direct mechanisms, such as oxidative damage and gonadal toxicity, and indirect mechanisms, such as endocrine disruption [33]. Some trace elements, while necessary in small amounts, can exert toxic effects when concentrations exceed physiological thresholds. Additionally, certain metals, such as

lead and cadmium, are endocrine-disrupting compounds that interfere with hormonal pathways and can alter reproductive health [34].

3.4 Evidence of elemental exchange between sperm and seminal plasma

Essential elements such as calcium, magnesium, potassium, sodium and zinc are present in seminal plasma, predominantly in the ionic form. Spermatozoa use supecialized ion channels and pumps to regulate intracellular and extracellular concentrations, ensuring optimal osmotic balance, pH, and membrane potential for motility and fertilization [35]. This dynamic exchange parallels that between erythrocytes and plasma in blood [36,37], albeit on a smaller scale due to the lower cellular density in semen.

In reproductive technology, the composition of semen simulants (artificial seminal plasma) injected into the vaginal environment is calibrated carefully to mimic the physiological levels of essential elements as closely as possible [38]. This is performed to optimise the conditions for sperm function and fertilisation.

The distinction between intracellular and extracellular metal homeostasis highlights the precision with which these elements are regulated to support fertilisation. For example, zinc, potassium, calcium and magnesium concentrations in seminal plasma often significantly exceed those found in serum. Conversely, other elements such as copper and iron are consistently higher in the serum than in the seminal plasma [8,17,39] (Table 2). These differential patterns underscore the highly specialised microenvironment of the male reproductive tract, which is tailored to the needs of spermatozoa during their journey through the male and female reproductive systems [40]. The choice of whether to measure whole blood or serum (in the case of blood), and whole semen or seminal plasma (in the case of semen), depends on study objectives, exposure profiles, and the need to differentiate chronic from acute or localized effects.

4. Analytical approaches in seminal plasma metallomics

4.1 A key analytical technique: advantages and limitations

Among the various methodologies for comprehensive profiling of both essential and ultratrace metals in seminal plasma, ICP-MS provides high sensitivity, a broad dynamic range, and the ability to measure multiple elements simultaneously [41]. These features make it especially suitable for the comprehensive profiling of both

essential and ultratrace metals in seminal plasma. However, ICP-MS requires meticulous calibration and strict contamination control. Sample digestion protocols, which often employ nitric acid and hydrogen peroxide, are critical for obtaining consistent measurements [42].

4.2 Comparison with other methods

Although atomic absorption spectrometry remains a standard technique for single-element analysis, its throughput and sensitivity to ultratrace levels can be limited. Inductively coupled plasma optical emission spectrometry (ICP-OES) also offers multi-element capabilities by measuring the light emitted from excited atoms or ions in the ionized gas plasma (not to be confused with seminal plasma), but it generally provides higher detection limits (i.e., lower sensitivity) compared to ICP-MS, which detects ions based on their mass-to-charge ratio [16]. Given the wide range of metal concentrations in seminal plasma, ICP-MS has become the method of choice for advanced metallomic studies.

4.3 Sampling and pre-analytical considerations

The proper collection and storage of seminal plasma samples are of paramount importance, as metal contamination can arise from containers or spermatozoa. Centrifugation to remove sperm, spermatogenic cells and other cellular and particulate debris [43] precedes storage at −80 °C in several protocols [44].

4.4 Data interpretation and quality control

Metals in seminal plasma often exhibit non-Gaussian distribution, and researchers should apply nonparametric statistics or data transformations to handle skewed data [45]. Moreover, external reference materials to analytical quality control for seminal plasma are scarce, which necessitates the reliance on serum-based or in-house calibrations [46,47].

5. Fractionation of the ejaculate: prostate vs. seminal vesicle contributions

5.1 Normal physiology of ejaculation and fraction dominance

Semen primarily consists of secretions from the prostate and seminal vesicles, with minor contributions from other sources, such as the bulbourethral glands,

epididymides and the testes. Understanding each gland's contribution is crucial for assessing male reproductive health [48,49]. Theoretically, prostatic fluid can be obtained through prostatic massage [50], and seminal vesicular fluid is collected via aspiration under transrectal ultrasound guidance [51]. However, these methods are associated with significant invasiveness and a high risk of contamination, limiting their application to specialized contexts, such as pharmacokinetic studies or the evaluation of obstructive azoospermia in cases of male infertility. Given these challenges, there remains a clear need for less invasive and more precise techniques to evaluate the gland-specific contributions to semen [52].

Split ejaculation sampling, which involves collecting multiple fractions from a single ejaculation, is often employed as a noninvasive method to evaluate the dynamics of accessory gland secretions in vivo [53]. This sampling technique leverages the physiological property that approximately the first 30% of the ejaculate typically originates from the prostate, while the remaining two-thirds are primarily composed of seminal vesicular fluid [54]. Observational studies using transrectal ultrasound have further demonstrated that the timing of prostatic contractions differs from that of seminal vesicle contractions by at least several seconds [55]. Additionally, prostatic fluid is typically watery, while seminal vesicular fluid has a gel-like consistency [56,57]. This difference in texture can serve as a helpful indicator of whether the ejaculate has been successfully fractionated during sampling.

5.2 Biochemical and elemental differences in early vs. subsequent fractions

Fractionation studies in the 1970s showed that the initial portion of the ejaculate, dominated by prostatic fluid, generally has higher sperm concentration and motility, whereas seminal vesicular fluid contains only a small number of sperm [58]. Subsequent research further demonstrated that excessive exposure to seminal vesicular fluid can reduce sperm motility, shorten lifespan, compromise nuclear chromatin stability, and negatively affect sperm DNA integrity [59].

Chemically, the first fraction tends to have elevated levels of elements, such as zinc, calcium and magnesium, reflecting prostatic secretion [53,56]. In contrast, the subsequent fraction is often more voluminous and enriched with phosphorus and arsenic [8,53] (Table 2). Notably, it was already recognized in the 1990s that seminal vesicular fluid may contain prostaglandins, semenogelins, and other factors potentially capable of reducing sperm motility [60,61]. In fact, significant progress has been made in elucidating the functions of numerous bioactive substances

present in seminal vesicular fluid, and their contributions to sperm function regulation and modulation of the immune environment in the female reproductive tract are increasingly being understood [49,62]. However, further research is required to comprehensively clarify the interactions of newly identified components and their physiological significance [63].

In addition, the use of split ejaculation sampling for trace element studies poses specific methodological challenges. Potential pitfalls include incomplete separation of fractions, cross-contamination between the initial and subsequent fractions, and an increased likelihood of contamination arising from multiple collection containers. Consequently, ensuring the reliability and accuracy of research designs employing this approach requires careful timing to capture the intermittent outflow from the urethra, as well as the standardization of protocols—including the use of low-contamination consumables. By addressing these factors, split ejaculation sampling can remain a valuable tool for elucidating gland-specific trace element distributions in semen.

5.3 Clinical implications of fractionation for fertility assessment

Elucidating the secretory profiles of each accessory gland offers valuable insights into male fertility. In addition to testicular factors, the etiology of semen abnormalities also involves post-testicular contributors—namely epididymal and accessory gland functions—which remain relatively underexplored [64]. Seminal plasma is considered an optimal resource for investigating these factors because it reflects the local pathophysiology of the male reproductive organs [65]. A practical approach proposed more than half a century ago revealed that using only the initial fraction of ejaculate for IUI could result in higher pregnancy rates [66]. Similarly, the "withdrawal coital method", wherein the initial fraction is ejaculated intravaginally while the remaining portion is expelled outside the vagina, can be viewed as an early technique that harnesses the distinct physiological effects of prostatic and seminal vesicular secretions on sperm to improve pregnancy outcomes [67].

From a diagnostic perspective, specific biomarkers in seminal plasma have long been used to evaluate glandular function. Zinc was identified in the 1980s as a marker of prostatic secretion [68], while fructose was used to assess seminal vesicular function [69]. By the 2000s, the use of prostate-specific antigen (PSA) in seminal plasma to evaluate prostatic secretory capacity had also been reported [52,70]. Other established markers of prostatic activity include citric acid,

γ-glutamyl transpeptidase, and acid phosphatase [71,72]. Additionally, parameters such as pH and viscosity have been proposed as potential indicators of seminal vesicular dysfunction [73].

Zinc, calcium and magnesium are well-known trace elements predominantly found in prostatic fluid [53]. Moreover, our previous research demonstrated that a wide range of elements—including lithium, sodium, sulfur, manganese, iron, cobalt, copper, zinc, selenium, rubidium, strontium, molybdenum, cesium, barium and thallium—are predominantly present in prostatic fluid [8]. In contrast, only two trace elements, phosphorus and arsenic, appear to be more concentrated in seminal vesicular fluid [8].

Building on earlier studies, which proposed combining fructose and PSA measurements to simultaneously quantify the relative contributions of the prostate and seminal vesicles to total semen volume [52], we have introduced a novel approach. Using ICP-MS to measure a broad spectrum of trace elements [16], we demonstrated its analytical advantage in assessing the imbalance between prostatic and seminal vesicular secretions. This method could serve as a foundation for developing superior diagnostic strategies. In addition, our earlier findings suggest that certain trace elements are maintained at higher or lower concentrations in seminal plasma than in serum, potentially reflecting active regulation by epithelial cells in the prostate or seminal vesicles [8]. Although the fundamental physiological rationale for maintaining divergent levels in seminal plasma remains unclear, the fact that seminal plasma-to-serum ratios vary significantly depending on the specific element indicates their potential value as biomarkers for evaluating post-testicular factors [17].

Overall, fractionation not only reveals the distinct biochemical signatures contributed by the prostate and seminal vesicles but also has meaningful clinical relevance. By understanding which glandular functions are compromised or exaggerated, targeted therapeutic interventions may be devised, such as adjusting supplementation to enhance prostatic support or addressing potential excess seminal vesicular components. Although epididymal fluid represents less than 10% of the total ejaculate volume [52], it remains pivotal for sperm maturation, with neutral α-glucosidase and L-carnitine serving as recognized functional markers [74,75]. However, no specific trace element has yet been definitively linked to epididymal fluid, and it is unclear whether subtle variations in epididymal secretion significantly affect metallomic profiles. Empirical or supplemental therapies, including coenzyme

Q10, vitamins, zinc and selenium, continue to be studied for their potential to improve sperm quality in men with unexplained male infertility [76]. Moreover, measuring seminal plasma biomarkers may identify subgroups of idiopathic oligoasthenoteratospermic men who could benefit from L-carnitine supplementation [75], suggesting a new avenue for personalized treatment approaches. Future work may clarify how post-testicular, epididymal and accessory gland contributions jointly influence male reproductive outcomes and guide more targeted interventions.

6. Environmental and occupational exposures to trace elements

6.1 Seminal plasma as a sensitive biomarker of environmental exposure

Although blood and urine are conventional biomarkers, seminal plasma can be more specific for reproductive outcomes. High levels of certain toxicants in seminal plasma may cause infertility before overt changes appear in the blood [77]. This specificity stems from the role of accessory glands in excreting or concentrating certain metals [31].

6.2 Regional pollution and male infertility

Regions such as Campania (Italy) and Opole (Poland) have been associated with industrial contamination and diminished sperm quality [78,79]. Similar associations have been noted in heavily industrialised parts of India and China, underscoring how local environmental factors can shape seminal plasma metallomic profiles [13].

6.3 Regulatory thresholds and gaps in knowledge

Despite the accumulating evidence, no universally accepted threshold values exist for "toxic" vs. "safe" levels of elements in seminal plasma. Establishing reference intervals for multiple metals remains a challenge [12], and researchers must disentangle chronic low-dose exposure, which may exert subtle but significant effects, from acute high-dose exposure.

7. Emerging trends: personalized medicine and machine learning

7.1 Clustering and other data-driven approaches in metallomics

The ultimate goal of seminal plasma biomarker research in addressing male infertility is achieving partner pregnancy. To this end, two potential case-control study designs can be envisioned. One approach involves comparing fertile and infertile men, while the other focuses on examining differences in biomarker profiles to predict pregnancy outcomes in their partners.

Both approaches, however, face limitations when relying on traditional statistical methods. This is due to the known correlations—both positive and negative—among the concentrations of seminal plasma biomarkers that reflect accessory gland secretory functions. For example, positive correlations have been reported between zinc and citric acid concentrations, while negative correlations exist between zinc and fructose concentrations [80]. Additionally, several studies have documented consistent positive correlations among heavy metals in seminal plasma [79,81–86].

Given these complexities, machine learning techniques have emerged as promising tools in seminal plasma metallomics research. Specifically, studies comparing fertile and infertile men have utilized supervised machine learning methods, such as Bayesian kernel machine regression and weighted quantile sum models, to address the intricate multicollinearity among trace elements [86]. These approaches have demonstrated efficacy in distinguishing between fertile and infertile groups when clear classification is achievable. However, real-world challenges remain, including difficulty in definitively excluding female infertility as a factor, the possibility of spontaneous conception in men initially categorized as infertile, and the relatively large sample sizes required for supervised learning methods.

To overcome these challenges, we explored the use of unsupervised machine learning techniques to maximize the utility of pilot data obtained from a relatively small-scale study [8]. This approach analyzed high-dimensional elemental data, aiming to identify latent subtypes within a population of male patients with infertility —defined as individuals whose fertility status cannot be clearly categorized as fertile or infertile. By clustering these patients based on the ratios of key elements in seminal plasma to serum, we identified distinct patterns reflecting “prostatic fluid dominance” and “seminal vesicular fluid dominance”. Our findings suggest that the subgroup characterized by “prostatic fluid dominance” may be associated with better pregnancy outcomes.

7.2 Integration with sperm analysis

The DNA fragmentation index, recognized as an advanced biomarker, holds promise as a robust predictor of fertilization outcomes [87]. By integrating metallome profiles as a third pillar alongside traditional semen parameters, such as sperm concentration, motility and morphology, as well as advanced biomarkers, such as DNA fragmentation indices, we anticipate a more powerful framework for addressing the complex mechanisms of infertility. This integrative approach not only has the potential to enhance diagnostic precision but may also guide targeted therapeutic strategies, including nutritional supplementation and avoidance of specific toxicant exposure, thus paving the way for more effective interventions in the future.

7.3 Challenges and future directions

Large-cohort studies, prospective designs and standardised sample handling are essential to validate these approaches. The high cost and limited accessibility of ICP-MS remain a barrier; moreover, as the reference intervals for seminal plasma metals remain poorly defined, clinicians must interpret results with caution.

8. Conclusions

Seminal plasma metallomics studies highlight how inorganic elements shape male reproductive health. This field, galvanised by improvements in ICP-MS and machine learning, expands our understanding beyond semen analysis alone. Early versus subsequent fraction patterns, environmental exposure and personalised therapies converge to form a new paradigm in infertility research. Robust prospective studies with standardised protocols are required to validate and translate these findings into clinical practice.

Availability of data and materials

Not applicable.

Author contributions

KK and TO—designed the research study. KK and TTan—performed the experiments. MU, KY, AI, HNe, TTak, TK and HNi—provided assistance and advice. KK, TTan, AN

and DN—analysed the data. KK and TO—wrote the manuscript. All authors contributed to the editorial changes in the manuscript. All the authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare no conflict of interest.

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