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Article

Evaluation of Inductively Coupled Plasma Mass Spectrometry for Determining Ca, Cu, Fe, Mg, Mn, Se and Zn in Bovine Semen Samples using a Simple Sample Dilution Method

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Um método simples e rápido para a determinação de Ca, Cu, Fe, Mg, Mn, Se e Zn em sêmen bovino por espectrometria de massas com plasma indutivamente acoplado (q-ICP-MS) é descrito. Previamente as análises, 200 µL de amostras foram diluídas 1:50 em solução contendo Triton® X-100 (0,01% v/v) e ácido nítrico (0,5% v/v). Os limites de detecção foram de 0,3, 0,03, 0,2, 0,04, 0,04, 0,03 e 0,03 µg L^{-1} para ^{44}Ca, ^{63}Cu, ^{57}Fe, ^{24}Mg, ^{64}Zn, ^{82}Se e ^{55}Mn, respectivamente. Para efeitos de comparação e validação do método, quatro amostras de sêmen bovino foram analisadas por ICP-MS pelo método proposto e por espectrometria de absorção atômica com chama (FAAS) ou espectrometria de absorção atômica em forno de grafite (GF AAS), e não foram encontradas diferenças estatísticas entre as técnicas com aplicação do teste-*t* (95% de confiança). Então, o método proposto foi aplicado na determinação de Ca, Cu, Fe, Mg, Mn, Se e Zn em amostras de sêmen bovino coletadas de diferentes raças, as quais são usadas em programas de reprodução animal e inseminação artificial.

A simple and fast method for the determination of Ca, Cu, Fe, Mg, Mn, Se and Zn in bovine semen by quadrupole inductively coupled plasma spectrometry (q-ICP-MS) is described. Prior to analysis, samples (200 µL) were diluted 1:50 in a solution containing 0.01% v/v Triton® X-100 and 0.5% v/v nitric acid and directly analyzed by ICP-MS. The limits of detection of the method are 0.3, 0.03, 0.2, 0.04, 0.04, 0.03 and 0.03 µg L^{-1} for ^{44}Ca, ^{63}Cu, ^{57}Fe, ^{24}Mg, ^{64}Zn, ^{82}Se and ^{55}Mn, respectively. For purposes of comparison and method validation, four ordinary bovine semen samples were directly analyzed by ICP-MS and by flame atomic absorption spectrometry (FAAS) or graphite furnace atomic absorption spectrometry (GF AAS), with no statistical difference between the techniques at the 95% level when applying the *t*-test. Then, the proposed method was applied in the determinations of Ca, Cu, Fe, Mg, Mn, Se and Zn in collected samples of bovine semen from different breeds, which are used in reproduction programs and artificial insemination.

Keywords: inductively coupled plasma mass spectrometry, atomic absorption spectrometry, bovine semen, sample dilution, essential elements

Introduction

Semen is an organic fluid that is secreted by the gonads (sexual glands) and other male sexual organs. The components of semen come from two sources: sperm and seminal plasma. The seminal plasma contains a complex range of organic constituents such as proteins, fructose, flavins and inorganic constituents. The interest in the effect of metals on the reproductive cells has led to several studies to determine metals in both human and

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animal semen.[1-3] Zinc serves to help to stabilize the DNA-containing chromatin in sperm cells. Zinc deficiency may result in lowered fertility because of the increased sperm fragility and can also adversely affect spermatogenesis.[4] Copper deficiency shows relation to the production of smaller volumes of ejaculate, lower sperm concentration, lower motility and morphological changes. The oxidative stress is identified as a major cause of infertility in mammals, and elements such as copper, iron, selenium and zinc are present in enzymes with antioxidant activity.[5,6] Iron is an essential component of some proteins that are present in Leydig and Sertoli cells.[6] Selenium is related to the sperm maturation in the epididymis and the supply of ATP (adenosine-5'-triphosphate), that is essential for testicular development and spermatogenesis.[7] Studies showed that calcium has a key role in the progressive motility of sperm, it can bind to calmodulin and this complex regulates the activity of several enzymes that are related to the motility.[4,8] Manganese is a component of a metalloenzyme that is linked to the synthesis of steroids and increased release of gonadal hormones.[9] Another crucial element in the physiology of spermatozoa is magnesium, which is present in high concentrations in semen and is required in almost all enzyme systems. This element has an important role in the spermatogenesis, especially in relation to the sperm motility and ejaculation.[4,8]

Thus, the analysis of metals in semen is critical in the investigation of disorders that are related to the animal or male reproductive system. The analysis makes possible to directly evaluate the concentrations of the elements of interest in the reproductive cells and seminal fluid, which can determine the semen quality and fertility success.[8,10-14] The semen analysis is also useful in the evaluation of the exposure to toxic metals in the environment.[1,6]

The analysis of trace elements in biological samples is predominantly performed using atomic spectrometry techniques, such as atomic absorption spectrometry (AAS),[15-23] inductively coupled plasma emission spectrometry (ICP-OES)[24] and inductively coupled plasma mass spectrometry (ICP-MS).[25-29] ICP-MS offers several advantages, including simultaneous multielement measurement capability coupled with very low limits of detection, a wider linear dynamic range, allowing the determination of major and trace elements at the same sample injection and isotopic information.

The sample preparation is always a critical step for the chemical element determination in biological samples prior to analysis by atomic spectrometric techniques. In the case of semen, all of the found methods in the literature involve the sample digestion in acid medium to eliminate the organic matter, resulting in increased analysis time, risk of sample contamination and loss of volatile elements. Alternatively, to simplify the matrix, many studies have used the seminal plasma (the liquid part of semen that is composed of secretions from the accessory glands) which is obtained by semen centrifugation.[8,10-14] However, the concentration of metals in seminal plasma does not directly express their concentration in semen.[30]

The purpose of the present study was to establish and validate a fast method for determining seven essential elements in bovine semen based on a simple sample dilution prior to ICP-MS analysis. Results were compared with those from atomic absorption spectrometry methods. Further application of the validated procedure to the analysis of collected bovine semen from animals of different breeds was also provided.

Experimental

Reagents

All used reagents were of analytical-reagent grade (Sigma, St Louis, MO, USA), except HNO_3 (Sigma, St Louis, MO, USA) which was previously purified in a Kürner quartz sub-boiling still before use. High purity deionized water (resistivity of 18.2 mΩ cm) from a Milli-Q water purification system (Millipore, Bedford, MA, USA) was used throughout. All solutions were stored in high-density polyethylene bottles. Plastic bottles were cleaned by soaking in 15% (v/v) HNO_3 for 24 h, rinsed five times with Milli-Q water and dried in a class 100 laminar flow hood before use. A clean laboratory and a class 100 laminar flow hood were used for preparing the solutions.

For analysis using graphite furnace atomic absorption spectrometry (GF AAS), stock solutions of Se, Cu and Mn containing 1000 mg L^{-1} of each element (Merck, Darmstadt, Germany) and the chemical modifier palladium (Pd), (Fluka, Buchs, Switzerland), a solution at 1% v/v and magnesium ($Mg(NO_3)_2$) (Fluka, Buchs, Switzerland), at 0.05% v/v were used. Analytical calibration standards were daily prepared over the range of 2-10 μg L^{-1} for Cu and 2.5-25 μg L^{-1} for Mn by suitable serial dilutions of each stock solution in 0.5% v/v HNO_3. Se was analyzed by standard addition, range 0-20 μg L^{-1}. The selected modifier was a mixture of 20 μg palladium and 5 μg magnesium nitrate, which was injected after the sample and before proceeding with the drying step.

For the analysis by flame atomic absorption spectrometry (FAAS), stock solutions of Ca, Fe, Mg and Zn containing 1000 mg L^{-1} of each element (Ultra Scientific, North Kingstown, USA) and lanthanum chloride ($LaCl_3 \cdot 7H_2O$) releasers (Sigma, St Louis, MO, USA) 5% m/v solutions

were used. Analytical calibration standards were daily prepared over the range of 2.5-5.0, 0.04-0.12, 0.10-0.20 and 0.06-0.14 mg L^{-1} for Ca, Fe, Mg and Zn, respectively, by suitable serial dilutions of each stock solution in 0.5% v/v HNO_3. Calcium, Mg and Zn were determined by matrix matching using a pool of bovine semen diluted 1:10 for analysis of Ca and Zn and diluted 1:50 for the determination of Mg. The used diluent was nitric acid at 0.5% v/v. The iron was determined using calibration in aqueous solution, HNO_3 0.5% v/v. For all the samples, 200 µL of standard solution of lanthanum chloride releasers was added, for a final concentration of 0.1% v/v.

For the ICP-MS method, multielement stock solutions containing 1000 mg L^{-1} of Ca, Cu, Fe, Mg, Mn, Se and Zn were obtained from Perkin-Elmer (PerkinElmer, Norwalk, CT, USA) and analytical calibration standards were daily prepared over the range of 200-2000 µg L^{-1}. The standards were prepared by suitable serial dilutions of stock solution in 0.01% v/v Triton® X-100 and 0.5% v/v HNO_3. The calibration curves for digested samples were prepared by suitable serial dilutions of stock solution in 4% v/v HNO_3. Rhodium, iridium and yttrium were used as an internal standard at the concentration of 10 µg L^{-1}. The internal standard was diluted from 1000 mg L^{-1} stock standard (PerkinElmer, Norwalk, CT, USA). For calibration by matrix matching, it was used a pool of diluted bovine semen 1:50 in 0.01% v/v Triton® X-100 and 0.5% v/v HNO_3 and two blank samples (*i*) containing the matrix, diluent and internal standard and (*ii*) the reagent blank (which contained only diluent and internal standard). The analyte content in the base bovine semen was considered and discounted.

Instrumentation

Graphite furnace atomic absorption spectrometric method

Selenium, copper and manganese were determined in bovine semen using an AAS 5 atomic absorption spectrometer (Zeiss, Germany). Deuterium-arc background correction was employed to correct for non-specific absorption. All measurements were performed using integrated absorbance (s). Hollow cathode lamps for Se, Cu and Mn (Analytik Jena, Germany) were operated at 15, 15 and 20 mA with a spectral bandwidth of 1.0, 0.7 and 0.2 nm, respectively. The selected wavelengths were 196.0, 324.8 and 279.5 nm for Se, Cu and Mn, respectively. Argon 99.996% (White Martins, São Paulo, SP, Brazil) was used as a protective and purge gas. Pyrolytic graphite coated polycrystalline electrographite tubes with total pyrolytic graphite platforms (Zeiss) were used throughout. 20 µL of diluted sample were directly deposited onto the L'vov platform. The heating programs are given in Table 1. The modifier was a mixture of 20 µg palladium and 5 µg magnesium nitrate, which was injected after the sample and before proceeding with the drying step.

Table 1. Optimal heating programs for determining Se, Cu and Mn in bovine semen by GF AAS

Temperature / °C	Ramp / (°C s^{-1})	Hold / s	Gas flow rate / (mL min^{-1})
90	5	20	300
105	3	20	300
110	2	10	300
1000[a] / 1200[b,c]	250	10	300
1900[a] / 2000[b,c]	1500	4	0
2500	500	4	300

[a]Se; [b]Cu; [c]Mn.

Flame atomic absorption spectrometric method

Calcium, iron, magnesium and zinc were determined in bovine semen by using an AA 6200 atomic absorption spectrometer (Shimadzu, Japan). Deuterium lamp background correction was employed to correct the non-specific absorption. Hollow cathode lamps for Ca, Fe, Mg and Zn (Hamamatsu Photonics, Japan) were operated at 10, 12, 8 and 8 mA with a spectral bandwidth of 0.7, 0.2, 0.7 and 0.7 nm and gas flow rates of 2.0, 2.2, 1.8 and 2.0, respectively. The selected wavelengths were 422.7, 248.3, 285.2 and 213.9 nm for Ca, Fe, Mg and Zn, respectively.

The inductively coupled plasma-mass spectrometry method

An ELAN DRC II ICP-MS instrument (Perkin Elmer, USA) was used to determine the elements in bovine semen. This instrument was used in the standard mode. The ICP-MS was operated with Pt sampler and skimmer cones purchased from Perkin Elmer. Argon 99.999% (White Martins, São Paulo, SP, Brazil) was used. The optimized instrument conditions and measurement parameters are listed in Table 2.

For the experiments with digested bovine semen samples, an ETHOS Start D microwave oven (Milestone, USA) was used. The operating conditions are shown in Table 3.

Specimen collection

Forty-one bovine semen samples were collected from healthy bulls of different breeds using an artificial vagina. The samples were obtained from a company specializing in reproduction programs and artificial insemination, packed in metal-free conical tubes (15 mL), and kept in liquid nitrogen from the time of collection until delivery

Table 2. ICP-MS operating conditions

Perkin Elmer Elan DRC II	
Spray chamber	Cyclonic
Nebulizer	Meinhard®
RF power / W	1100
Ar nebulizer gas flow / (L min^{-1})	0.56-0.98 (daily optimized)
Measures	
Scan mode	Peak hopping
Replicate time / s	1
Dwell time / ms	50
Sweeps *per* reading	40
Integration time / ms	2000
Replicates	3
Investigated isotopes	^{44}Ca, ^{63}Cu, ^{57}Fe, ^{24}Mg, ^{55}Mn, ^{82}Se and ^{64}Zn

Table 3. Heating program of the microwave oven used for digestion of bovine semen samples analyzed by ICP-MS

Step	time / min	Power / W	Temperature / °C
1	10	450	140
2	20	750	190
3	30	–	cooling

of the samples, after which the samples were kept in a freezer (–80 °C) until analyses. These whole samples were used to estimate background levels for essential metals in bovine semen.

For the development of analytical methodology, four different bovine semen pools were prepared using sperms that were collected from six ordinary animals.

Preparation of bovine semen samples for direct introduction

To evaluate the calibration method in aqueous medium and by matrix-matching, the samples (200 µL) were diluted 1:50 in a solution containing 0.01% v/v Triton® X-100 and 0.5% v/v HNO_3. Ir, Rh and Y were added as internal standards to all samples to get a final concentration of 10 µg L^{-1}. The blank samples were prepared in the same way.

Preparation of bovine semen samples for digestion

When the digestion procedure was used for the bovine semen sample analysis, 1 mL of sample (pool of bovine semen) and 7 mL of 20% v/v nitric acid were mixed in a PFA digestion vessel. Then, the samples were digested in a microwave oven according to the program described in Table 3. After the digestion, the samples were transferred to 15 mL conical tubes (Falcon®, BD, USA) and the volume was made up to 10 mL with deionized water. Ir, Rh and Y were added as internal standards to get a final concentration of 10 µg L^{-1}. The blank samples were prepared in the same way.

Preparation of bovine semen samples for atomic absorption spectrometry analysis

For GF AAS analysis, each pool of bovine semen samples (200 µL) was diluted 1:10 in a solution containing 0.5% v/v HNO_3. Cu and Mn were analyzed against aqueous standards and Se was analyzed by the standard addition method. The blank samples were prepared in the same way as the standard solutions.

For FAAS analysis, samples (1 mL, pool of bovine semen) were diluted 1:10 to determine Ca, Fe and Zn and 1:50 to determine Mg in a solution containing 0.5% v/v HNO_3 and 0.1% v/v lanthanum chloride as releasers. Matrix-matching calibration was used for Ca, Mg and Zn determinations. On the other hand, Fe was analyzed using calibration in aqueous medium.

Results and Discussion

Selection of the dilution factor and diluent

In the last few years, the determination of chemical elements in biological matrices using direct introduction of the sample to the ICP-MS analysis has become more common. However, to the best of our knowledge, there is no method which directly introduces bovine semen samples to ICP-MS.

For direct sample introduction, the selection of the dilution factor of clinical specimens before the introduction of the sample to ICP-MS is a critical point. A simple 1:10 sample dilution before the analysis by quadrupole inductively coupled plasma spectrometry (q-ICP-MS) can minimize the effects from the matrix. However, this dilution factor may cause impregnation of the matrix in the nebulization chamber and the deposition on the nebulizer and cones, resulting in interference that limits the analysis of large numbers of samples. The system may also need to be cleaned more frequently. In addition, higher sample volumes need to be collected. Thus, dilution factors of 1:20 and 1:50 were evaluated.

Another type of interferences during the ICP-MS measurements is the polyatomic interfaces, which occur due to the formation of ionized molecules with the same nominal mass of the analyte in study. These polyatomic ions are generally produced from the argon and other gases,

used reagents in the treatment of samples or components of the matrix. The most common polyatomic interferences occur due to the formation of oxides, double charge ions, dimers, hydrides and hydroxides. Since the formation of ions in the plasma depends on the carrier gas flow and the RF power, these parameters were properly adjusted to minimize these interferences.

Initially, it was evaluated the possibility for directly determine the metals in bovine semen using a solution containing 0.01% v/v Triton® X-100 and 0.5% v/v nitric acid as the diluent. This selection is based on previous publications of our group and others, showing the successful use of this diluent for the direct analysis of clinical samples (urine and blood) by ICP-MS.[26,31,32] Moreover, the dilution in Triton® X-100 improves the repeatability of the analytical signals due to the high viscosity of the sample.

Preliminary experiments with four pools of bovine semen (analyzed in triplicate) show that in different sample dilution conditions (1:10, 1:20 and 1:50), the obtained results with the respective precision (relative standard deviation, RSD) were similar (always better than 10%). For elements with possible interference, more than one isotope was evaluated. The ICP-MS parameters, such as RF power and Ar nebulizer gas, were also evaluated and the optimized conditions are expressed in Table 2. For the subsequent experiments, it was chosen to dilute the bovine semen samples 1:50 with a solution containing 0.01% v/v Triton® X-100 and 0.5% v/v nitric acid.

Evaluation of the homogeneity of the sample aliquots

The random distribution of spermatozoa, even when the sample is well mixed, accounts for most of the variability or lack of precision in bovine semen analysis results. The use of pipettes can significantly underestimate semen volume due to residual fluid being left behind in the pipette. For this reason, it was checked possible variations in precision due to the viscosity of bovine semen samples. For this study, two pools of samples were left for 15 min until liquefaction and then, vortexed for 2 min. From each pool, 5 different aliquots of 200 µL of sample were pipetted and mixed (at least three times with the pipette) with 9800 µL of the diluent solution (0.5% v/v HNO_3 + 0.01% v/v Triton® X-100) in a 15 mL conical tube. Each of these diluted aliquots was analyzed in triplicate and the uncertainties of the measurements were calculated for each analyte in order to test the homogeneity of the sample aliquots and possible errors during the sampling. For all analytes, the uncertainties were lower than 5%. This demonstrates that a sampling volume of 200 µL is a representative volume of the total bovine semen sample.

Determination of metals in bovine semen by ICP-MS with direct sample introduction

Two methods of calibration were evaluated: (*i*) calibration against aqueous solution and (*ii*) calibration against matrix-matching. The results of the direct sample introduction procedure were also compared with the results that were obtained after the digestion of bovine semen. For this experiment, 3 bovine semen pools (see material and methods section) were used. The results are presented in Table 4 and are expressed as the average of triplicate readings of three samples from each evaluated pool of bovine semen with their respective standard deviation, along with the element. The statistical evaluation (one way ANOVA, confidence level of 95%) showed that the obtained results by the direct sample introduction (calibration in aqueous medium or by matrix-matching) and after the digestion of samples

Table 4. Comparison between direct sample introduction and microwave sample digestion for the determination of trace elements in bovine semen samples by q-ICP-MS. Results expressed in µg L^{-1} (Cu and Mn) and mg L^{-1} (Ca, Fe, Mg, Zn and Se)

Analyte	Aqueous medium calibration			Matrix-matching			Microwave digestion		
	Sample (bovine semen pool, n = 3), concentration ± SD								
	1	2	3	1	2	3	1	2	3
Ca	33 ± 0.1	38 ± 0.2	35 ± 0.9	33 ± 0.9	38 ± 0.3	32 ± 0.3	39 ± 0.8	38 ± 0.8	40 ± 0.6
Cu	70 ± 2	90 ± 0.4	72 ± 1	62 ± 2	81 ± 4	56 ± 1	71 ± 1	90 ± 0.3	67 ± 1
Fe	0.6 ± 0.02	0.6 ± 0.06	0.7 ± 0.05	0.4 ± 0.02	0.4 ± 0.02	0.6 ± 0.01	0.5 ± 0.04	0.5 ± 0.07	0.5 ± 0.01
Mg	9 ± 0.3	7 ± 0.05	8 ± 0.01	8 ± 0.02	5 ± 0.02	8 ± 0.03	8 ± 0.02	5 ± 0.05	8 ± 0.07
Zn	1.2 ± 0.03	1.0 ± 0.01	1.2 ± 0.02	1.2 ± 0.03	0.9 ± 0.03	1.0 ± 0.01	1.5 ± 0.01	1.3 ± 0.08	1.3 ± 0.08
Mn	46 ± 2	40 ± 0.1	49 ± 1	45 ± 2	40 ± 2	47 ± 0.1	55 ± 1	44 ± 3	50 ± 3
Se	0.6 ± 0.02	0.6 ± 0.03	0.5 ± 0.01	0.7 ± 0.03	0.6 ± 0.02	0.5 ± 0.01	0.8 ± 0.08	0.6 ± 0.08	0.5 ± 0.09

SD: standard deviation.

were in good agreement. This indicates that the proposed procedure with direct sample introduction of bovine semen samples and analysis by q-ICP-MS is probably free of interference. The method could be appropriately validated by analyzing standard reference materials. However, no such reference materials are commercially available. Thus, to check the accuracy and validate our method, the use of atomic absorption spectrometry was further evaluated as a comparative technique for the determination of Cu, Mn, Se, Ca, Fe, Mg and Zn. However, before bovine semen analysis by GF AAS, parameters such as pyrolysis and atomization curves were optimized.

Optimization of pyrolysis and atomization curves for the determination of Cu, Mn and Se by GF AAS

The curves were obtained with a 20 µL injection of a bovine semen pool diluted at 1:10 in 0.5% v/v HNO_3. The selected modifier was a mixture of 20 µg palladium and 5 µg magnesium nitrate which was injected after the sample and before proceeding with the drying step. Results are shown in Figure 1. Similar thermal behavior was also observed for the standards in aqueous medium. The optimized heating program shown in Table 1 was used to determine analytes in bovine semen using GF AAS with calibration against aqueous solution.

Comparison between ICP-MS and AAS for determining chemical elements in bovine semen samples

A comparison between the proposed method for the determination of Ca, Cu, Fe, Mg, Mn, Se and Zn in four samples of bovine semen (four pools of semen) by using inductively coupled plasma mass spectrometry and Cu, Mn and Se or Ca, Fe, Mg and Zn determination by GF AAS and by FAAS, respectively, in bovine semen samples is described in Table 5. The proposed ICP-MS method with direct sample introduction and calibration against aqueous medium showed results for the determination of the elements Ca, Cu, Fe, Mg, Mn, Se and Zn in bovine semen that were similar to those obtained by FAAS and GF AAS. Student's *t*-test showed no statistical differences at the 95% level, demonstrating the accuracy of the proposed method.

As shown in Table 6, the limits of detection (LOD) of the proposed ICP-MS method were 0.3, 0.03, 0.2, 0.04, 0.04, 0.03 and 0.03 µg L^{-1} for ^{44}Ca, ^{63}Cu, ^{57}Fe, ^{24}Mg, ^{64}Zn, ^{82}Se and ^{55}Mn, respectively. The LOD values of the method for the determination of Cu, Mn and Se by GF AAS were 2.5, 1.4 and 1.6 µg L^{-1}, respectively, and for the determination of Ca, Fe, Mg and Zn by FAAS, the LOD were 60, 12, 5.3 and 8.7 µg L^{-1}, respectively. Typical within-day precision was < 9% (n = 4), while between-day

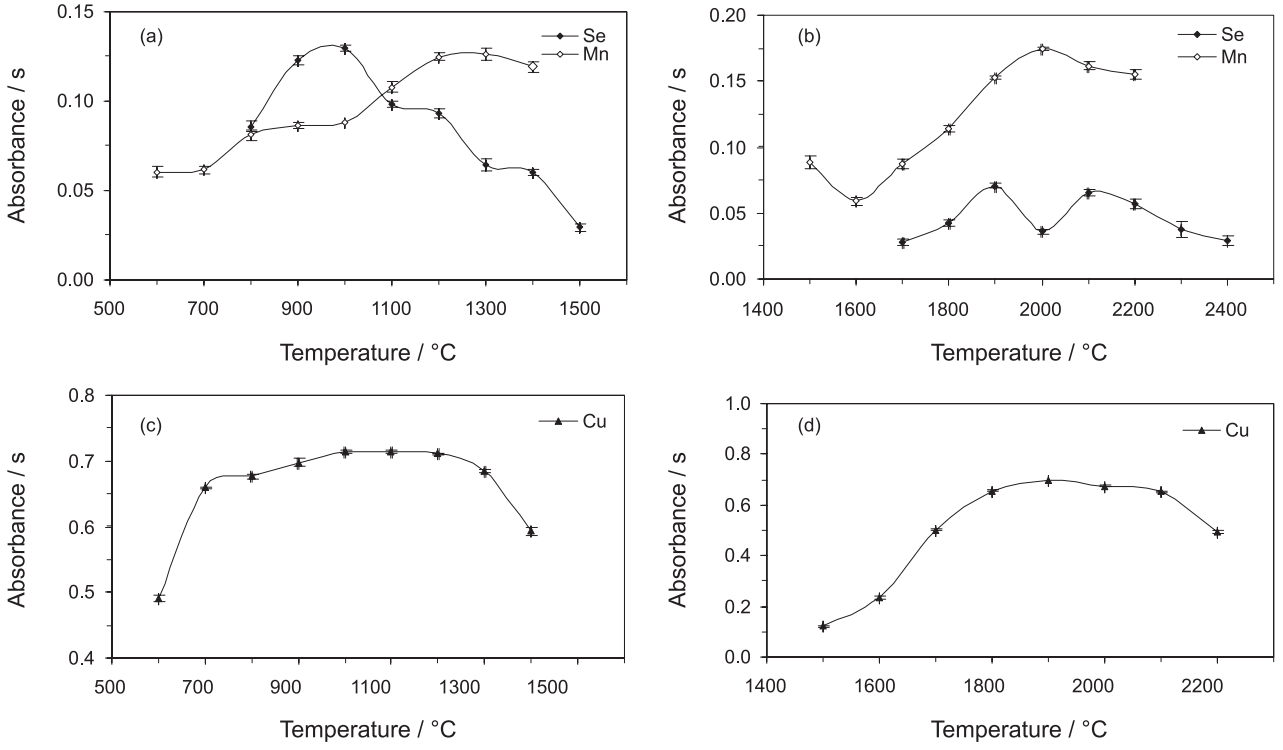

Figure 1. Optimization of experimental conditions for the GF AAS determination of Se, Mn and Cu in bovine semen samples: (a) pyrolysis curves for Se and Mn, (b) atomization curves for Se and Mn, (c) pyrolysis curve for Cu and (d) atomization curve for Cu. Injection of 20 µL of bovine semen diluted 1:10 in 0.5% v/v HNO_3 and 10 µL of chemical modifier (20 µg palladium and 5 µg magnesium nitrate).

Table 5. Analysis of bovine semen samples by ICP-MS and AAS, results expressed in μg L^{-1} (Cu and Mn) and mg L^{-1} (Ca, Fe, Mg, Zn and Se)

Analyte	Analytical technique							
	ICP-MS				AAS			
	Sample (bovine semen pool, n = 3), concentration ± SD				Sample (bovine semen pool, n = 3), concentration ± SD			
	1	2	3	4	1	2	3	4
Ca	33 ± 0.1	38 ± 0.2	35 ± 0.9	29 ± 0.5	33 ± 0.4[a]	41 ± 0.5a	38 ± 0.7[a]	26 ± 0.7[a]
Cu	70 ± 2	90 ± 0.4	72 ± 1	86 ± 2	66 ± 2[b]	94 ± 0.2[b]	73 ± 2[b]	88 ± 1[b]
Fe	0.6 ± 0.02	0.6 ± 0.06	0.7 ± 0.05	0.6 ± 0.02	0.6 ± 0.03[a]	0.6 ± 0.02[a]	0.6 ± 0.04[a]	0.7 ± 0.02[a]
Mg	9 ± 0.3	7 ± 0.05	8 ± 0.01	9 ± 0.2	9 ± 0.08[a]	8 ± 0.1[a]	8 ± 0.2[a]	9 ± 0.1[a]
Zn	1.2 ± 0.03	1.0 ± 0.01	1.2 ± 0.02	1.1 ± 0.02	1.2 ± 0.05[a]	1.1 ± 0.05[a]	1.3 ± 0.03[a]	1.2 ± 0.05[a]
Se	0.6 ± 0.02	0.6 ± 0.03	0.5 ± 0.01	0.6 ± 0.01	0.7 ± 0.06[b]	0.6 ± 0.2[b]	0.5 ± 0.02[b]	0.5 ± 0.07[b]
Mn	46 ± 2	40 ± 0.1	49 ± 1	53 ± 1	49 ± 5[b]	46 ± 1[b]	49 ± 1[b]	53 ± 1[b]

[a]Analysis by FAAS; [b]analysis by GF AAS; SD: standard deviation.

Table 6. Limits of detection (LOD) determined by ICP-MS and AAS

Analyte	Analytical technique	
	ICP-MS LOD / (μg L^{-1})	AAS LOD / (μg L^{-1})
Ca	0.3	60[a]
Cu	0.03	2.5[b]
Fe	0.2	12[a]
Mg	0.04	5.3[a]
Zn	0.04	8.7[a]
Se	0.03	1.6[b]
Mn	0.03	1.4[b]

[a]Analysis by FAAS; [b]analysis by GF AAS.

precision was < 14% RSD (n = 3) for all analytes with the proposed ICP-MS procedure.

Application to the proposed method for the analysis of bovine semen used in artificial insemination programs

The proposed method was then applied for determining Ca, Cu, Fe, Mg, Zn, Se and Mn in bovine semen samples from animals of different classes (zebu and European beef cattle and zebu and European dairy cattle) and breeds that are used in reproduction and artificial insemination programs. The results are shown in Table 7. One-way ANOVA shows that a similarity exists between the concentrations of Ca, Cu, Fe, Zn, Se and Mn in bovine semen samples from animals of different classes, p-value > 0.05. However, this was not observed for Mg since the concentrations were significantly lower in zebu beef cattle and higher in European dairy cattle. Unfortunately, it was not identified an explanation for that.

Conclusions

To our knowledge, this paper is the first to propose the determination of Ca, Cu, Fe, Mg, Zn, Se and Mn in bovine semen samples by ICP-MS. The obtained results by direct sample introduction (calibration in aqueous medium or by matrix-matching) and after digestion of samples are in good agreement and indicate that the proposed procedure is free of interference. Moreover, the simple and fast procedure that is proposed here with direct introduction of diluted

Table 7. Determination of elements in bovine semen samples by ICP-MS, results expressed in μg L^{-1} (Cu and Mn) and mg L^{-1} (Ca, Fe, Mg, Zn and Se)

Analyte	Breed (concentration ± SD (n = 3))			
	Zebu beef catle (n = 18)	European beef catle (n = 9)	Zebu dairy catle (n = 11)	European dairy catle (n = 3)
Ca	39 ± 17	50 ± 12	38 ± 9	43 ± 19
Cu	61 ± 34	58 ± 12	57 ± 27	56 ± 14
Fe	0.5 ± 0.2	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.05
Mg	9 ± 2	12 ± 3	12 ± 2	12 ± 3
Zn	1.0 ± 0.5	0.9 ± 0.3	1.0 ± 0.5	1.0 ± 0.3
Se	0.6 ± 0.2	0.7 ± 0.1	0.8 ± 0.2	0.7 ± 0.06
Mn	33 ± 8	26 ± 14	30 ± 14	29 ± 11

Results are expressed as concentration, average of three replicates of the samples ± standard deviation (SD).

bovine semen samples to the ICP-MS (dilute-and-shoot procedure) considerably reduces the time of analysis and risks of sample contamination. The ICP-MS method was validated by comparison with atomic absorption spectrometry methodologies. Comparing to AAS, the use of ICP-MS allows the determination of the seven elements in study at the same sample injection. For the proposed method, it was observed a slight variation of the signal for analysis (RSD < 5%) and an analytical frequency average of 25 samples *per* h (200 samples in 8 h), which is very attractive for routine analysis. Moreover, in general, the precision was better for the ICP-MS method when compared to AAS methodologies.

As an additional study, the proposed procedure was applied to the analysis of bovine semen that is used in artificial insemination programs to establish reference ranges.

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