Brief communication

Trace metals in embryo and cell culture, and handling media products including hyaluronidase enzyme, mineral oil and pure water

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Abstract

Background
It is known that trace metals play a crucial role in health and disease. In view of the critical roles of trace metals in cell and embryo culture media, and due to the paucity of information on the presence or absence of trace elements in cell and embryo handling and culture media, we estimated and determined the presence and concentration of some trace metals in a number of media utilized for handling and culture of embryos and cells.
Materials and methods
Different culture and handling media were utilized with and without the addition of 5% serum proteins. Other handling media such as mineral oil, hyaluronidase and PVP were investigated without addition of protein-supplements. The investigations were performed using inductively coupled plasma mass spectrometry (ICP-MS). The levels of Arsenic (As), Cobalt (Co), Copper (Cu), Iron (Fe), Selenium (Se) and Zinc (Zn) were determined in the various media obtained from the manufacturers.
Results
All trace metals investigated were not present in all media tested, with the significant exception of iron. Iron was detected in most media tested. Arsenic was detected only in hyaluronidase.
Discussion and Conclusion
This study has demonstrated that the trace metals investigated, such as cobalt, copper, selenium, and zinc, are not present in all the media tested with the exception of iron which was present in most media and arsenic was detected in hyaluronidase. The serum supplement used in culture media could be the main source of trace metals.

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Introduction

Metal ions make up some of the trace elements. Ten of the approximately 20 basic elements that the human body needs to function effectively are metal elements. According to what is known at this time, metals like Na, K, Mg, Ca, Fe, Mn, Co, Cu, Zn, and Mo are necessary for life and must be present in the right amounts for good health (see review by Zoroddu et al., 2019).

A metal is thought to be present in about half of all proteins (Thomson et al., 1998). According to a different estimate, between 25 and 30 percent of all proteins are thought to need metals to function (Waldron et al., 2009) In addition to serving as cofactors for enzymes, metalloproteases have a variety of roles in cells, including protein transport and storage, signal transduction, and the prevention of infectious illnesses (Carver 2013). Given that even synthetic proteins without an evolutionary background are able to bind metals, it is possible that the abundance of metal-binding proteins is a property of the amino acids that

proteins use (Wang et al., 2018). The last four references on metalloproteins in this paragraph were adapted from Wikipedia.

It is generally known that trace metals play a crucial role in health and disease, and their imbalance in nutrition is hazardous (see reviews by Vithanage et al., 2019; Chochia et al., 2020). It is common knowledge that trace metals serve as cofactors. The nutritional solutions known as cell and embryo culture media are used to support the growth of cells and embryos *in vitro*. The quality of the cells and embryos produced in the culture media will be impacted by an imbalance, a deficiency, or an excess of trace metals. Uncontrolled trace metal content in media has clear adverse implications. The composition of embryo culture media was previously reported by Morbeck et al. (2014, 2017; and Tarahomi et al. (2019).

There is, however, a dearth of information regarding the concentrations of some significant trace metals that may be present in current embryo handling and culture media, including cell handling and culture media. Although their significance in human reproduction (Lewicka et al., 2017), male reproduction (Mirnamniha et al., 2019), and female reproduction (Garnier et al., 2021) has been reviewed in part, the importance of trace metals in embryo and cell handling and culture media has not been seriously investigated.

In view of the critical roles of trace metals in cell and embryo culture media, and due to the paucity of information on the presence or absence of trace elements in cell and embryo handling and culture media, we planned to estimate the presence and concentrations of some critical and common trace metal in a number of media utilized for handling and culture of cella and embryos. The trace elements in the embryo and cell handling and culture media were not intentionally added to the mixture but are present in these media as contaminants.

Due to limitation of resources we could only estimate the concentrations of a few trace elements, namely, Arsenic (As), Cobalt (Co), Copper (Cu), Iron (Fe), Selenium (Se) and Zinc (Zn) in cell and embryo handling and culture media, including pure water, hyaluronidase enzyme preparation, mineral oil and PVP (polyvinylpyrrolidone).

Materials and Methods

Different culture media, including mineral oil as well as other handling media such as hyaluronidase and PVP, were obtained from their respective manufacturers. Some media that were not supplemented with serum proteins by the manufacturers were measured with and without the addition of 5% serum proteins (HSA or FSA; Table 1). These two serum supplements were added to the respective media in keeping with the norms of cell and embryo culture practices.

Table 1: The Media investigated and preparation

1	Irvine® Medium w/o HSA, USA	5 ml med
2	Irvine® Medium + HSA, USA	4.75 med+ 250µl HSA
3	Global® Culture Media, USA	5ml med
4	Pure Water	5 ml med
5	Sage® Medium+ HSA, USA	4.85 med+ 150 µl HSA
6	Sage® Medium+ w/o HSA, USA	5 ml med
7	Sage® Hepes Medium, USA	5 ml med
8	MEM Sigma Chemicals® USA	5 ml med
9	MEM+ FSA Sigma Chemicals® USA	5 ml med
10	MEM+ Hepes Sigma Chemical® USA	5 ml med
11	EBSS Sigma Chemicals® USA	4.75 med+ 250 µl HSA
12	EBSS+ FSA Sigma Chemicals® USA	5 ml med
13	EBSS+ Hepes Sigma Chemicals® +FSA	4.75 med+ 250 µl FSA
14	Cellcura® Embryo Culture Medium, Norway	5 ml med
15	Cellcura® Handling medium+ Hepes, Norway	5 ml med
16	IUI Medium Cellcura® Norway	5 ml med
17	Flushing Medium Cellcura®, Norway	5 ml med
18	Hyaluronidase Cellcura® , Norway [Custom-Manufactured by FertiPro, Belgium, (CMFB)]	1 ml of Hyal+ 4 ml pure water
19	PVP Cellcura®, Norway (CMFB)	1 ml of PVP+ 4 ml pure water
20	Mineral Oil Cellcura® Norway (CMFB)	3ml oil + 7ml pure water
21	Mineral Oil Sigma® USA	3 ml oil+ 7 ml pure water

*Cellcura® is now known as Synbios® media (www.synbiosmedia.com)

Table 2a: Trace metals in cell and embryo culture and handling media (in ppb)

S. No	Media	Preparation	Parts per billion (ppb)		
			Fe	As	Co, Cu, Zn, Se
1	Irvine Culture Medium w/o HSA	5 ml med	2.52	Not detected	Not detected
2	Irvine Culture Medium+ HSA	4.75 med+ 250µl HSA	34.82	Not detected	Not detected
3	Global Culture Medium	5ml med	27.12	Not detected	Not detected
4	Pure Water	5 ml med	Not detected	Not detected	Not detected
5	Sage Culture Medium+ HSA	4.85 med+ 150 µl HSA	18.46	Not detected	Not detected
6	Sage Culture Medium+ w/o HSA	5 ml med	Not detected	Not detected	Not detected
7	Sage Medium+ Hepes	5 ml med	Not detected	Not detected	Not detected
8	MEM (Minimum Essential Med)	5 ml med	0.63	Not detected	Not detected
9	MEM+ FSA	5 ml med	37.69	Not detected	Not detected
10	MEM+ Hepes	5 ml med	0.94	Not detected	Not detected
11	EBSS (Earle's Basal Salt Soln)	4.75 med+ 250 µl HSA	Not detected	Not detected	Not detected
12	EBSS+ FSA	5 ml med	37.25	Not detected	Not detected
13	EBSS+ Hepes	4.75 med+ 250 µl FSA	0.2	Not detected	Not detected
14	Cellcura Culture Medium	5 ml med	0.38	Not detected	Not detected
15	Cellcura Medium+ Hepes	5 ml med	0.32	Not detected	Not detected
16	IUI Medium Cellcura	5 ml med	0.38	Not detected	Not detected
17	Flushing Medium Cellcura	5 ml med	2.79	Not detected	Not detected
18	Hyaluronidase Cellcura	1 ml of Hyal+ 4 ml pure water	0.97	2.44	Not detected
19	PVP Cellcura	1 ml of PVP+ 4 ml pure water	0.35	Not detected	Not detected
20	Mineral Oil Cellcura	3ml oil + 7ml pure water	0.48	Not detected	Not detected
21	Mineral Oil Sigma	3 ml oil+ 7 ml pure water	0.79	Not detected	Not detected

Table 2b: Trace metals in cell and embryo culture and handling media (ug/ml)

S. No	Media	Preparation	ug/ml		
			Fe	As	Co, Cu, Zn, Se
1	Irvine Culture Medium w/o HSA	5 ml med	0.00050	Not detected	Not detected
2	Irvine Culture Medium+ HSA	4.75 med+ 250µl HSA	0.00690	Not detected	Not detected
3	Global Culture Medium	5ml med	0.00542	Not detected	Not detected
4	Pure Water	5 ml med	Not detected	Not detected	Not detected
5	Sage Culture Medium+ HSA	4.85 med+ 150 µl HSA	0.00369	Not detected	Not detected
6	Sage Culture Medium+ w/o HSA	5 ml med	Not detected	Not detected	Not detected
7	Sage Medium+ Hepes	5 ml med	0.00000	Not detected	Not detected
8	MEM (Minimum Essential Med)	5 ml med	0.00013	Not detected	Not detected
9	MEM+ FSA	5 ml med	0.00754	Not detected	Not detected
10	MEM+ Hepes	5 ml med	0.00019	Not detected	Not detected
11	EBSS (Earle's Basal Salt Soln)	4.75 med+ 250 µl HSA	Not detected	Not detected	Not detected
12	EBSS+ FSA	5 ml med	0.00745	Not detected	Not detected
13	EBSS+ Hepes	4.75 med+ 250 µl FSA	0.00004	Not detected	Not detected
14	Cellcura Culture Medium	5 ml med	0.00008	Not detected	Not detected
15	Cellcura Culture Medium+ Hepes	5 ml med	0.00006	Not detected	Not detected
16	IUI media cell culture	5 ml med	0.00008	Not detected	Not detected
17	Hyaluronidase Cellcura	1 ml of Hyal+ 4 ml pure water	0.00019	0.00049	Not detected
18	PVP Cellcura	1 ml of PVP+ 4 ml pure water	0.00007	Not detected	Not detected
19	Flushing Medium Cellcura	5 ml med	0.00056	Not detected	Not detected
20	Mineral Oil Cellcura	3ml oil + 7ml pure water	0.00005	Not detected	Not detected
21	Mineral Oil Sigma	3 ml oil+ 7 ml pure water	0.00008	Not detected	Not detected

The investigations were performed using inductively coupled plasma mass spectrometry (ICP-MS) in collaboration with the Department of Chemistry, Faculty of Science, University of Malaya, as per standard procedures.

The levels of arsenic, cobalt, copper, iron, selenium, and zinc were determined in the various media obtained from the manufacturers. Only one replication was possible due to limited resources.

Results

The results of the study are given in Tables 2a and 2b. All trace metals investigated were not present in all media tested, with the significant exception of iron. Iron was detected in most media tested. Iron was not detected in pure water, EBSS, and the two Sage HEPES and Sage culture media. All these four media were devoid of added serum protein supplements. Arsenic was only detected in hyaluronidase enzyme medium.

Discussion

This study has demonstrated that the trace metals investigated, namely, cobalt, copper, selenium, and zinc, are not present in all the media tested. The only trace metal found in detectable quantities in the various media investigated was iron. The amount of iron in media supplemented with serum supplements is much higher than in their respective media that are devoid of added protein supplements. This would suggest that serum supplements could be the main source of trace metals in culture media. Of interest is the presence of arsenic in hyaluronidase enzyme preparation. The implication of the presence of arsenic in hyaluronidase is not known at this moment. Hyaluronidase is extracted from bovine testes which may explain the presence of arsenic in the enzyme preparation. Workers in therapeutic IVF must bear in mind this important finding because excess trace metals could find their way into the culture media if the quality of serum supplements are not carefully controlled and maintained. However, the limitation of this study is that procedures were not replicated due to a lack of material resources. This study may be informative, but it can only be considered preliminary at this stage.

In human reproduction, a lack of trace elements and metals can cause fetal development abnormalities and predispose a child to disorders later in life. Trace elements are the key elements of complex enzymes responsible for modulating the antioxidant defense system of the organism. Optimum levels of trace elements are essential for antioxidant function. Trace elements are components of numerous regulatory enzymes and hormones that are essential to the division and differentiation of cells and embryos, as well as their further development. Mineral deficiencies are known to cause birth defects of the central nervous system and growth disorders. It thus follows that the same could be initiated or occur during the in vitro development of embryos in culture media with deficient levels or an imbalance of trace metals (Lewicka et al., 2017; Mirnamniha et al., 2019; Garnier et al., 2021). Service providers and patients will benefit from further research into the optimal requirements for trace metals in embryo culture media.

Conclusion

This study has demonstrated that the trace metals investigated, such as cobalt, copper, selenium, and zinc, are not present in all the media tested with the exception of iron which was present in most media. Arsenic was detected in hyaluronidase preparation. Hyaluronidase enzyme is extracted from bovine testes. The serum supplement used in culture media could be the main source of trace metals.

References

Carver PL (2013). "Chapter 1. Metal Ions and Infectious Diseases. An Overview from the Clinic". In Sigel A, Sigel H, Sigel RK (eds.). Interrelations between Essential Metal Ions and Human Diseases. Metal Ions in Life Sciences. Vol. 13. Springer. pp. 1–28. doi:10.1007/978-94-007-7500-8_1. ISBN 978-94-007-7499-5. PMID 24470087.

Chochia A, Geladze N, Gogberashvili K, Khachapuridze N, Bakhtadze S. [Modern Insues Of Essential Trace Elements Level Influence On

Children And Adulth Health (Review)]. Georgian Med News. 2020 May;(302):105-108. Abstract. Russian.

Garner TB, Hester JM, Carothers A, Diaz FJ. Role of zinc in female reproduction. Biol Reprod. 2021 May 7;104(5):976-994. doi: 10.1093/biolre/ioab023.

Lewicka I, Kocyłowski R, Grzesiak M, Gaj Z, Oszukowski P, Suliburska J. Selected trace elements concentrations in pregnancy and their possible role - literature review. Ginekol Pol. 2017;88(9):509-514. doi: 10.5603/GP.a2017.0093.

Mirnamniha M, Faroughi F, Tahmasbpour E, Ebrahimi P, Beigi Harchegani A. An overview on role of some trace elements in human reproductive health, sperm function and fertilization process. Rev Environ Health. 2019 Dec 18;34(4):339-348. doi: 10.1515/reveh-2019-0008.

Tarahomi M, Vaz FM, van Straalen JP, Schrauwen FAP, van Wely M, Hamer G, Repping S, Mastenbroek S. The composition of human preimplantation embryo culture media and their stability during storage and culture. Hum Reprod. 2019 Aug 1;34(8):1450-1461. doi: 10.1093/humrep/dez102.

Thomson AJ, Gray HB (1998). "Bioinorganic chemistry" (PDF). Current Opinion in Chemical Biology. 2 (2): 155–158. doi:10.1016/S1367-5931(98)80056-2. PMID 9667942.

Vithanage M, Kumarathilaka P, Oze C, Karunatilake S, Seneviratne M, Hseu ZY, Gunarathne V, Dassanayake M, Ok YS, Rinklebe J. Occurrence and cycling of trace elements in ultramafic soils and their impacts on human health: A critical review. Environ Int. 2019 Oct;131:104974. doi: 10.1016/j.envint.2019.104974.

Waldron KJ, Robinson NJ (January 2009). "How do bacterial cells ensure that metalloproteins get the correct metal?". Nature Reviews. Microbiology. 7 (1): 25–35. doi:10.1038/nrmicro2057. PMID 19079350. S2CID 7253420.

Wang, MS; Hoegler, KH; Hecht, M (2019). "Unevolved De Novo Proteins Have Innate Tendencies to Bind Transition Metals". Life. 9 (8): 8. doi:10.3390/life9010008

Zoroddu MA, Aaseth J, Crisponi G, Medici S, Peana M, Nurchi VM. The essential metals for humans: a brief overview. J Inorg Biochem. 2019 Jun;195:120-129. doi: 10.1016/j.jinorgbio.2019.03.013