ijlpr 2020; doi 10.22376/ijpbs/lpr.2020.10.5.L138-144

International Journal of Life science and Pharma Research

Research Article

Medicine for novel therapy

Level of Elements Present in Normal Human Seminal Plasma: A Study and Review

Sivapriya Narayanan[1] , Pandiyan Nadarajan[2], Skandhan P Kalanghot[3*] and Manasa Nallagatla[4]

[1]Post Graduate Student; [2]Professor; Chettinad Hospital and Research Institute, Kelambakkam 603 103, India

[3]Visiting Professor; [4] Graduate Student; Shri BMK Ayurveda Mahavidyalaya and Research Institute, Belagavi 590 001, India

Abstract: Childlessness remains as a problem with the couple who suffer and they are psychologically and socially very much disturbed Husband, wife or both are likely to be responsible for infertility. Male infertility is on rise world over. Many group of workers are on this study now. Detailed study on semen shall give the exact clue to male fertility status. Other than semen parameters, the biochemistry of semen secured attention of several group of workers. Knowledge in inorganic chemistry of semen –limited. Similar studies are in progress at different parts of the world. It is now known increase or decrease of some elements in semen lead to changes in sperm motility or semen quality. In relation to this, we tried to understand more about elements in semen. Considering electrolytes and metals are important for cell functions, few workers are involved in inorganic chemistry of semen study. The present study will improve the existing knowledge on elements in semen. Semen samples were supplied by 10 young, normal, healthy men. Samples were collected after maintaining an abstinence of five days. Collection was done around 9 am to exclude any chrono biological influence. From each sample, seminal plasma was separated at 1 and 3 hours and stored in deep freezer. After thawing and processing, samples were analyzed for elements in it using Inductively Couple Plasma Mass Spectrometry (ICPMS). A total number of 28 elements were found in all samples. Their values differed between 1 and 3 hour, either more or less. Study concludes with supporting evidences showing the role of elements in sperm survival or function.

Keywords: Male infertility, Human semen, Semen elements.

***Corresponding Author**

Skandhan P Kalanghot, , Visiting Professor, Shri BMK Ayurveda Mahavidyalaya and Research Institute, Belagavi 590 001, India

Recieved On 06 October 2020
Revised On 23 November 2020
Accepted On 26 November 2020
Published On 07 December 2020

Funding This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors

Citation Sivapriya Narayan ,Pandiyan Nadarajan, Skandhan P Kalanghot, Manasa Nallagatla , Level of Elements Present in Normal Human Seminal Plasma: A Study and Review.(2020).Int. J. Life Sci. Pharma Res.10(5), L138-144 http://dx.doi.org/10.22376/ijpbs/lpr.2020.10.5.L138-144

1. INTRODUCTION

Infertility is a major concern to couple as well as their clinician. The psychological and social implication of the problem is tremendous on couple and at large they feel desperate. When a couple reports for infertility, clinician starts investigating both male and female partners. The detailed knowledge on male reproductive organs and its functions are not as rich as that of female. Some reports stated this gap as 30 years in knowledge.[1,2] These reports promoted several groups of workers to explore the details of male reproductive system and its function. We reported the minute structure of human epididymis[3], the inorganic elements present in male reproductive structure and in semen[4]. Male infertility rate is on rise. [5-14] World over the rate differs, may be due to different conditions like atmosphere, climate, food intake, pollution and other unknown causes. Environmental contamination due to industrial pollution is known to lower testicular function.[15- 22] Investigation for male partners included that of blood and semen. Semen acquired large attention as clinicians and researchers have been trying to locate unknown causes for male infertility. Interest was paid to organic and inorganic elements present in semen. Aim of the present study was to identify and measure the concentration of different inorganic elements present in normal human semen samples.

2. MATERIALS AND METHODS

The study was conducted after getting approval from Research Committee of the Institution and Institutional Ethical Committee of Chettinad Hospital and Research Institute (CHRI/11/16-11-2015). All glass wares used in this study were cleaned in nitric acid, followed by tap water, deionized water, double deionized water and made to dry at 80°C in laboratory hot air oven, air except measuring glass wares which were dried in laboratory by placing on filter paper. Prior to enrolment consent of volunteers was taken. Selected volunteers were healthy, free from any disease and they belonged to the age group of 21 to 30 years. They belonged to the local region and were from Tamil speaking community. Semen was collected from 10 normal male subjects. They collected semen samples after maintaining five days of abstinence. [23,24]. Patients who were on treatment on Ayurvedic or Unani systems of medicine were excluded as few of their medicines contained minerals and metals. [25] Semen samples were collected around 9 am into clean and dry glass containers with wide mouth provided from laboratory. Semen was examined as per WHO.[26] Total sperm count and percentages of sperm motility are considered as two major criteria in semen study. [27] After microscopic study each sample was divided equally into two (A and B). Sample A was centrifuged at 1 hour and sample B was centrifuged at 3 hour to separate seminal plasma. Care was taken not to break or destroy cells by centrifugation, (1500rpm for 10mins).[27] Separated seminal plasma was collected in micro centrifuge tubes and stored at -20°C. Whole semen sample directly when stored to frozen state lead to change in values of zinc. [26] The procedure for analysis deserves careful attention. In one study, when same samples were analyzed by using two different procedures for atomic absorption, spectrophotometry, results differed.[28] We proceeded as follows. After thawing a sample, 0.5µl was taken and to which 0.6 µl of nitric acid (HNO_3), 0.4µ l of hydrochloric acid (HCl), 0.2 µl of hydrogen peroxide (H_2O_2) and 2400µl of deionized water were added. The mixture was then dried at 200^{0}C to powder form with the help of a digital heater. Prior to analysis this was dissolved in 45 ml of deionized water. Elemental analysis of samples (A and B) was done by employing Inductively Coupled Plasma Mass Spectrometry (ICPMS) (Agilent 7500CE Series, Octapole Reaction System, CA, and United States of America) available at Dignity Centre, Saidapet, and Chennai. Analysis using the method was done to identify and measure level of total 41 elements.

3. STATISTICAL ANALYSIS

We have employed, IBM Statistical Package for the Social Sciences (SPSS) version 20 for statistical analysis.

4. RESULTS

A total number of 28 elements were detected and measured in seminal plasma (A and B). The average of each element was calculated and presented in Table 1. Eight of them niobium, palladium, platinum, tantalum, tellurium, tin, vanadium and zinconium were below detection level. The values of each element detected was different at 1 (A) and 3(B) hours (Table 1). The elements like antimony, arsenic, beryllium, boron, cadmium, cebium, germanium, lithium, mercury, scandium, selenium, silica and silver were not detected in samples (A and B) studied. Statistically, no difference was seen when values of the two groups were compared within and between them.

Table 1. Showing the levels of 28 elements at 1 hour and 3 hour

Elements	At One hour(µg/L)	At Three hour(µg/L)
Aluminum	37.3	53.3
Barium	10.3	13.2
Calcium	1646.3	1662.9
Cerium	379.6	197.8
Chromium	77.4	81.0
Cobalt	0.27	0.46
Copper	BDL	2.7
Gallium	0.2	BDL
Gold	2.58	0.91
Iron	376.0	425.2
Lead	10.36	1.12
Magnesium	762.6	744.3
Manganese	7.96	7.92
Molybdenum	0.66	0.54

Nickel	29.5	15.8
Niobium	BDL	BDL
Palladium	BDL	BDL
Platinum	BDL	BDL
Potassium	13250.0	11030.0
Sodium	22180.0	18820.0
Strontium	6.1	1.4
Tantalum	BDL	BDL
Tellurium	BDL	BDL
Tin	BDL	BDL
Titanium	8.4	5.6
Vanadium	BDL	BDL
Zinc	1772	1499
Zinconium	BDL	BDL

5. DISCUSSION

In human, several electrolytes and metals are detected in blood, hair [29,30] and sweat.[31] Semen elemental study gathered attention since the second half of last century.[32-39] The most important point in elemental study is preferring high quality instruments. In our studies we employed different instruments like Neutron Activation Analysis,[40] Mass Emission Spectroscope,[41] Direct Couple Plasma Emission spectroscope,[42] X-ray Diffraction, [43] Electron Microscopic Energy Dispersive X-ray Analysis[4] and Atomic Absorption Spectrophotometry. [22]In a detailed study, human male reproductive organs – testis, epididymis-caput, corpus and cauda-prostate gland, seminal vesicle, Cowper's gland and vas deferens were studied along with seminal plasma and spermatozoa pellet for trace elements. [44] Origin of different electrolytes and metals present in semen was identified by using split ejaculation technique.[44-48] Individual and within subject variability in semen element is also reported. [49] Several workers interested in the study of elements in semen reported the level of different elements like sodium, potassium, calcium, magnesium,[38,50,51] iron,[52] zinc,[39,53] copper,[54,55] cadmium, chromium, nickel and lead. [56] Manganese was reported in all normal samples. [56-58] Similarly, vanadium was present in all normal samples. [59] Cadmium, chromium, nickel and lead were not present in all samples. [56,57] Gold was detected in semen for the first time in 1981. [40] Some Investigators in this field were interested to find out if any change in level of these elements was present in pathological samples. They have studied and reported this in case of sodium, potassium, copper, zinc, gold, calcium, magnesium, and iron. [38-40,44,45,55,56,60-69] Volunteers of this study were advised to collect semen samples at 9 am. Fixed collection time is important. Studies showed samples collected at different timings differed in elemental level. As an example calcium and magnesium in semen was maximum at 00:00:00 hours and at this time the total sperm count and percentage of active motile spermatozoa were also maximum. [70-72] It is an important point on selection of instrument for elemental study. As an example we detected gold by employing Mass Emission Spectroscope. [41] But not by Direct Couple Plasma Emission Spectroscope. [42] Electron Microscopic Energy Dispersive X-Ray Analysis showed Gold inside and outside spermatozoa. [4] Further, X-Ray Diffraction study showed details of gold which remained in trimetal complex with zinc and copper. [43] In the present study we opted Inductively Couple Plasma Mass Spectrometry (ICPMS) for elemental study after processing advised for its usage. Processing method is an important part in elemental analysis. [73] In the present study twenty eight elements were identified and measured (Table 1). Increased or decreased level of elements from 1 to 3 hours (Table 1) , was statistically insignificant. This was due to the entry or exit of a particular element in sperms at the time of its separation from semen (at 1 or 3 hour). This was supported by earlier reports. [37,89,71] Each element may have a specific role to play in life of a sperm cell. [74] Results of studies conducted by different groups of workers proved this. The role of sodium, potassium, calcium and magnesium was like in other cells. [75,76] Some elements in semen are important for sperm motility. [71,77] Calcium is important for sperm capacitation, a crosomal reaction,[78,79] sperm motility,[80-82] metabolic function [82] and fertilization. [79] Magnesium in sperm is essential for release of energy and thus for motility. [81] Strontium is important for sperm capacitation.[83] Cadmium was not observed in this study (Table 1). Which was reported by others? [56,84] Each instrument has limitation in identifying elements mainly depending on its concentration. We preferred ICPMS in this study. Dawson et al. [85] reported the levels of lead, cadmium and aluminum maintained an inverse correlation with the percentage of live spermatozoa. Concentration of lead, manganese, chromium and nickel were reported. [56] Molybdenum decreased at 3 hr in semen (Table 1). Meeker et al. [86] reported this metal as a male reproductive toxicant. The level of lead decreased from 1 to 3 hr (Table 1). Pleban and Mei et al.[84] measured the level of lead. We did not detect lead in one of our earlier studies. [41] Selenium was not seen in the present study (Table 1). Selenium was studied in detail by Blein et al. [87] Increased level of elements in seminal plasma remain toxic to sperms. For example when experimentally micro amount of copper was added to normal semen, spermatozoa became immotile. [88] We observed decrease in level of gold from 1 to 3hr (Table 1). The element gold is proved as important for fertility. [89] Its role was known to Ayurveda, a traditional medicine, widely used in India and other Asian countries. In many cases of male infertility, physicians in Ayurveda prescribed gold in the form of its ash, "Suvarna Bhasma" (SB) (Gold Ash). The amount of gold present in it is known. [25] Clinical studies conducted by different groups of workers where SB prescribed to infertile men showed improvement in total sperm count and percentage of sperm motility. [90,91] Further, laboratory studies showed addition of micro amount of SB into semen increased the percentage of progressive motile sperms and their survival period. [69] Zinc level was high at 1 hour (Table 1). It is known mature sperms release zinc [35] and it is important for function of motility. [38,39,65,92] Whenever zinc level is low in seminal plasma, sperm remains motionless. [35,93] Semen elements remained inside and/or outside sperms [43,68] which are not likely to be equal on both sides of the membrane.

Calcium was more inside the sperm [2], which is present in head and flagellum. Different instruments were helpful to identify the location of elements in cells. Nine elements were identified in acrosome, nucleus and mid piece of sperm. [33] Zinc is essential for architectural organization of sperm [32] and maintenance of sperm membrane structure. [94] Summarizing the total study (Table 1), majority of reported elements are useful for the survival and/or functioning of spermatozoa. Further studies may prove usefulness of different elements present in semen.

6. CONCLUSION

In conclusion, in this study, by employing Inductively Couple Plasma Mass Spectrometry, we observed the presence of 28 elements in human seminal plasma and their values differed from 1 to 3 hour. This might be due to the entry or exit of a that particular element, depending on its essentiality in function of spermatozoa. Some elements function like in other cells. Study also showed some elements were below detection level maybe due to the less sensitivity of the instrument. More work is to be carried out by employing different analytical instruments to measure the exact level of few elements seen in this study as BDL. Similarly experimental studies are to be conducted to find out the effect of different elements on functions of sperm.

7. ACKNOWLEDGEMENT

Authors appreciate very much and are thankful to Dr. Sheik SSJ Ahmed and Dr. V Alexander of Chettinad Academy of Research and Education for their timely suggestions and help during the period of study.

8. AUTHORS CONTRIBUTION STATEMENT

Dr Pandiyan N and Dr Skandhan K P conceptualized the total work and Dr Sivapriya N gathered and analyzed the data. Dr Skandhan designed the manuscript; with Dr Sivapriya N and Dr Manasa; framed the final report, which was approved by all.

9. CONFLICT OF INTEREST

Conflict of interest declared none.

10. REFERENCES

1. World Health Organization. Technical Report Series NO : 582. The Epidemiology of Infertility, Switzerland; 1975.
2. Weyer RA. Male Contraception: will get it? Do we want it? Savvy. 1980;3:62-4.
3. Skandhan KP, Soni A, Joshi A, Avni KPS, Gupta BD. Human epididymis: structural pattern, total length and inner surface area. Urologia. 2017;84:215-7. doi: 10.5301/uj.5000229, PMID 28574145.
4. Skandhan KP, Sumangala B, Amith S, Avni KPS. Electron microscopic (energy dispersive X-ray analysis) study of human male reproductive organs and semen. Biol Trace Elem Res. 2011;141(1-3):91-5. doi: 10.1007/s12011-010-8723-0, PMID 20526753.
5. Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. Reprod Biol Endocrinol. 2015;13:37. doi: 10.1186/s12958-015-0032-1, PMID 25928197.
6. Chaitrali G, Santrupti K, Niranjan K, Skandhan KP, Sukumar N. Male infertility rate: A Retrospective study. Int J Infertile Fetal Med. 2019;10:1-3.
7. Collins HP, Kalisch D. The health of Australian males. Health Welf. 2011.
8. Jungwirth A, Giwercman A, Tournaye H, Diemer T, Kopa Z, Dohle G, Krausz C, European Association of Urology Working Group on Male Infertility. European Association of Urology guidelines on Male Infertility: the 2012 update. Eur Urol. 2012;62(2):324-32. doi: 10.1016/j.eururo.2012.04.048, PMID 22591628.
9. Kumar N, Singh AK. Trends of male factor infertility, an important cause of infertility: a review of literature. J Hum Reprod Sci. 2015;8(4):191-6. doi: 10.4103/0974-1208.170370, PMID 26752853.
10. Lunenfeld B, Van Steirteghem A, Bertarelli Foundation. Infertility in the third millennium: implications for the individual, family and society: condensed meeting report from the Bertarelli Foundation's second global conference. Hum Reprod Update Second Global Conference. 2004;10(4):317-26. doi: 10.1093/humupd/dmh028, PMID 15192057.
11. Mehra BL, Skandhan KP, Prasad BS, Pawankumar G, Singh G, Jaya V. Male infertility rate: a retrospective study. Urologia. 2018;85(1):22-4. doi: 10.5301/uj.5000254, PMID 28967062.
12. Skandhan KP, Mazumdar BN. High incidence of male involuntary infertility. Infertility. 1982;5:167-74.
13. Skandhan KP, Vadodaria H, Langalia D, Mazumdar BN. Incidence of male factor in involuntary infertility. Andrologia. 1982;14(4):328-30. doi: 10.1111/j.1439-0272.1982.tb02272.x, PMID 6889822.
14. Skandhan KP, Patel IA, Mehta YB. Rate of involuntary infertility among male partners. Ital J Med. 1986;2:71-8.
15. Assennato G, Paci C, Baser ME, Molinini R, Candela RG, Altamura BM, Giorgino R. Sperm count suppression without endocrine dysfunction in lead-exposed men. Arch Environ Health. 1987;42(2):124-7. doi: 10.1080/00039896.1987.9935808, PMID 3579367.
16. Babalola OO, Babajide SO. Selected heavy metals and electrolyte levels in blood of workers and residents of industrial communities. African J Bio Chem Rese. 2009;3:37-40.
17. Danadevi K, Rozati R, Reddy PP, Grover P. Semen quality of Indian welders occupationally exposed to nickel and chromium. Reprod Toxicol. 2003;17(4): 451-6. doi:10.1016/s0890-6238(03)00040-6, PMID 12849857.
18. Genuis SJ, Birkholz D, Rodushkin I, Beesoon S. Blood, urine, and sweat (BUS) study: monitoring and elimination of bioaccumulated toxic elements. Arch Environ Contam Toxicol. 2011 Aug 1;61(2):344-57. doi: 10.1007/s00244-010-9611-5, PMID 21057782.
19. Lancranjan I, Popescu HI, GAvănescu O, Klepsch I, Serbănescu M. IReproductive ability of workmen occupationally exposed to lead. Arch Environ Health. 1975;30(8):396-401. doi: 10.1080/00039896.1975.10666733, PMID 1155972.

20. Lerda D. Study of sperm characteristics in persons occupationally exposed to lead. Am J Ind Med. 1992;22(4):567-71. doi: 10.1002/ajim.4700220411, PMID 1442789.
21. Li H, Chen Q, Li S, Yao W, Li L, Shi X, Wang L, Castranova V, Vallyathan V, Ernst E, Chen C. Effect of Cr(VI) exposure on sperm quality: human and animal studies. Ann Occup Hyg. 2001;45(7):505-11. doi: 10.1016/S0003-4878(01)00004-7, PMID 11583652.
22. Morán-Martínez J, Carranza-Rosales P, Morales-Vallarta M, A Heredia-Rojas J, Bassol-Mayagoitia S, Denys Betancourt-Martínez N, M Cerda-Flores R. Chronic environmental exposure to lead affects semen quality in a Mexican men population. Iran J Reprod Med. 2013;11(4):267-74. PMID 24639755.
23. Skandhan KP. Can increase in frequency of ejaculation promote spermatogenesis? Med Hypo. 1989;30(2):81-2. doi: 10.1016/0306-9877(89)90089-3, PMID 2509860.
24. Skandhan KP, Skandhan S, Pandya CB, Shah RC. Daily ejaculates: level of zinc and copper in seminal plasma. Necessity for abstinence prior to semen collection. Infertility. 1985;8:279-91.
25. Prasad BS, Skandhan KP, Singh G. Analytical study of "Suvarna Bhasma" (gold ash), an ayurvedic medicine. Int J Drug Develop Technol. 2011;1:99-101.
26. World Health Organization. Laboratory manual for the examination and processing of human. Semen, Switzerland. 5th ed. 2010.
27. Skandhan KP, Mazumdar BN. Interdependence of count and motility of spermatozoa. Acta Eur Fertil. 1982;13(2):49-54. PMID 7186255.
28. Skandhan KP. Copper in seminal plasma: comparison of two processing methods for atomic absorption spectrophotometry. Arch Androl. 1986;16(3):243-5. doi: 10.3109/01485018608986947, PMID 3778021.
29. Schroeder HA, Nason AP. Trace metals in human hair. J Invest Dermatol. 1969;53(1):71-8. doi: 10.1038/jid.1969.110, PMID 5797518.
30. Takagi Y, Matsuda S, Imai S, Ohmori Y, Masuda T, Vinson JA, Mehra MC, Puri BK, Kaniewski A. Trace elements in human hair: an international comparison. Bull Environ Contam Toxicol. 1986;36(6):793-800. doi: 10.1007/BF01623585, PMID 3719115.
31. Cohn JR, Emmett EA. The excretion of trace metals in human sweat. Ann Clin Lab Sci. 1978;8(4):270-5. PMID 686643.
32. Baccetti B, Pallini V, Burrini AG. The accessory fibers of the sperm tail. II. Their role in binding zinc in mammals and cephalopods. J Ultrastruct Res. 1976;54(2):261-75. doi: 10.1016/s0022-5320(76)80155-4, PMID 814248.
33. Battersby S, Chandler JA. Correlation between elemental composition and motility of human spermatozoa. Fertil Steril. 1977;28(5):557-61. doi: 10.1016/s0015-0282(16)42557-4, PMID 852611.
34. Eliasson R. Accessory glands and seminal plasma with special reference to infertility as a model for studies on induction of sterility in the male. J Reprod Fertil Suppl. 1976;24(24);Suppl:163-74. PMID 794466.
35. Lindholmer C, Eliasson R. Zinc and magnesium in Human spermatozoa from different fractions of split ejaculates. Int J Fertil. 1974;19(1):45-8. PMID 4363157.
36. Lindholmer C, Eliasson R. In vitro release and uptake of zinc and magnesium by human spermatozoa. Int J Fertil. 1974;19(1):56-62. PMID 4363159.
37. Sheth AR, Rao SS. Potassium levels in Human Semen with reference to Sperm Motility. Experientia. 1962;18:324-5. doi: 10.1007/BF02151856, PMID 13911760.
38. Skandhan KP, Mehta YB, Chary TM, Archar MVS. Semen electrolytes in normal and infertility cases. I. Sodium, potassium, calcium and magnesium. J Obstet Gynaecol. 1978;28:278-85.
39. Skandhan KP, Skandhan S, Mehta YB. Semen electrolytes in normal and infertile subjects. II. Zinc. Experientia. 1978;34(11):1476-7. doi: 10.1007/BF01932363, PMID 720475.
40. Skandhan KP. Gold in human semen. Andrologia. 1981;13(1):78-82. doi: 10.1111/j.1439-0272.1981.tb00012.x, PMID 7258701.
41. Skandhan KP, Abraham KC. Presence of several elements in normal and pathological human semen samples and its origin. Andrologia. 1984;16(6):587-8. doi: 10.1111/j.1439-0272.1984.tb00417.x, PMID 6517364.
42. Skandhan KP, Pandya CB. Direct Couple plasma Emission Spectroscopic study on human seminal plasma. Adv Contr Dely Syst. 1986;2:256-7.
43. Skandhan KP, Amith S, Avni KPS. X-ray diffraction study on human male reproductive tract and semen. Urologia. 2009;76(3):198-202. doi: 10.1177/039156030907600305, PMID 21086292.
44. Skandhan KP. Importance of zinc in the semen quality of different fractions of split ejaculates. J Infertil. 1981;4:67-81.
45. Skandhan KP, Mazumdar BN, Sumangala B, Jaya V. Seminal plasma calcium in normal and infertile patients. Urologia. 2017;84(1):35-7. doi: 10.5301/uro.5000167, PMID 27647078.
46. Skandhan KP, Valsa J, Sumangala B, Jaya V. Level of copper in human split ejaculate. Urologia. 2017;84(1):51-4. doi: 10.5301/uro.5000178, PMID 27647080.
47. Valsa J, Gusani PH, Skandhan KP, Modi HT. Copper in split and daily ejaculates. J Reprod Med. 1994;39(9):725-8. PMID 7807487.
48. Valsa J, Skandhan KP, Khan PS, Sumangala B, Gondalia M. Split ejaculation study: semen parameters and calcium and magnesium in seminal plasma. Cent European J Urol. 2012;65(4):216-8. doi: 10.5173/ceju.2012.04.art7, PMID 24578965.
49. Mallidis C, Howard EJ, Baker HW. Variation of semen quality in normal men. Int J Androl. 1991;14(2):99-107. doi: 10.1111/j.1365-2605.1991.tb01071.x, PMID 1869320.
50. Eliasson R, Lindholmer C. Magnesium in human seminal plasma. Invest Urol. 1972;9(4):286-9. PMID 5058766.
51. Omu AE, Al-Bader AA, Dashti H, Oriowo MA. Magnesium in human semen: possible role in premature ejaculation. Arch Androl. 2001;46(1):59-66. doi: 10.1080/01485010150211164, PMID 11204619.
52. Skandhan KP, Mazumdar BN, Sumangala B. Study into the iron content of seminal plasma in normal and infertile subjects. Urologia. 2012;79(1):54-7. doi: 10.5301/RU.2012.9023, PMID 22328413.

53. Eliasson R, Lindholmer C. Zinc in human seminal plasma. Andrologie. 1971;3(4):147-53. doi: 10.1111/j.1439-0272.1971.tb01140.x.
54. Shaji G, Skandhan KP. Copper in human seminal plasma. Pan Minerva. Med. 1991;33:35-6.
55. Skandhan KP, Mazumdar BN. Semen copper in normal and infertile subjects. Experientia. 1979;35(7):877-8. doi: 10.1007/BF01955124, PMID 477836.
56. Skandhan KP, Makada MT, Amith S. Levels of cadmium, chromium, nickel, manganese and lead in normal and pathological human seminal plasma. Urologia. 2005;72(4):461-4. doi: 10.1177/039156030507200407.
57. Skandhan KP, Jiyo CS, Amith S. Different electrolytes and metals in human seminal plasma. Gazz Ital. 2007;166:181-6.
58. Li Y, Wu J, Zhou W, Gao E. Effects of manganese on routine semen quality parameters: results from a population-based study in china. BMC Public Health. 2012;12:919. doi: 10.1186/1471-2458-12-919, PMID 23107312.
59. D'Cruz OJ, Ghosh P, Uckun FM. Spermicidal activity of metallocene complexes containing vanadium(IV) in humans. Biol Reprod. 1998;58(6):1515-26. doi: 10.1095/biolreprod58.6.1515, PMID 9623614.
60. Bondani A, Aspeitia E, Aznar R, Gómez-Arzápalo E, Pascual C, Giner J. Correlation between sperm motility and electrolyte composition of seminal fluid in normal and infertile men. Fertil Steril. 1973;24(2):150-4. doi: 10.1016/s0015-0282(16)39498-5, PMID 4688103.
61. Gusani PH, Skandhan KP, Valsa C, Mehta YB. Sodium and potassium in normal and pathological seminal plasma. Acta Eur Fertil. 1988;19(6):333-6. PMID 3251391.
62. Jain V, Rai A, Misra S, Singh KM. Seminal gold content in healthy fertile men in India. Int J Ayurveda Res. 2010;1(3):172-4. doi: 10.4103/0974-7788.72490, PMID 21170210.
63. Skandhan KP. Zinc in normal human seminal plasma. Andrologia. 1981;13(4):346-51. doi: 10.1111/j.1439-0272.1981.tb00060.x, PMID 7283189.
64. Skandhan KP, Mazumdar BN. Correlation of sodium and potassium in human seminal plasma with fertilizing capacity of normal, and infertile subjects. Andrologia. 1981;13(2):147-54. doi: 10.1111/j.1439-0272.1981.tb00022.x, PMID 7247045.
65. Skandhan KP, Mazumdar BN. The relation between sodium and potassium in seminal plasma and the essentiality of these elements for sperm motility. Urologia. 1985;52(3):413-20. doi: 10.1177/039156038505200316.
66. Skandhan KP, Mazumdar BN. Zinc and copper in seminal plasma of normal and pathological samples: an analytical study. Urologia. 1986;53(2):200-8. doi: 10.1177/039156038605300207.
67. Skandhan KP, Skandhan S. Seminal plasma copper in fertile subjects and infertile patients. Adv Contr Dely Syst. 1987;3:231-6.
68. Skandhan KP, Sumangala B, Mehta YB, Roy PB, Amith S, Avni KPS. Level of gold in normal and pathological semen. Urologia. 2010;77(4):254-6. PMID 21234868.
69. Skandhan KP, Namboodiri E, Rao N, Prasad BS, Singh G. Effect of "Suvarna bhasma" (Gold Ash), an indigenous Ayurvedic preparation, on human sperm motility. J Sex Med. 2017;2:1009-13.
70. Valsa J, Skandhan KP, Gusani PH, Sahab Khan P, Amith S. Quality of 4-hourly ejaculates--levels of calcium and magnesium. Andrologia. 2013;45(1):10-7. doi: 10.1111/j.1439-0272.2012.01301.x, PMID 22540387.
71. Valsa J, Skandhan KP, Sumangala B, Jaya V. Time bound changes (in 24 h) in human sperm motility and level of calcium and magnesium in seminal plasma. Alex J Med. 2016;52(3):235-41. doi: 10.1016/j.ajme.2015.09.005.
72. Valsa J, Skandhan KP, Sumangala B, Amith S, Skandhan Avni KP. Effect of different timings of the day on semen and calcium and magnesium in it. Urologia. 2016;83(4):207-10. doi: 10.5301/uro.5000029, PMID 27516350.
73. Valsa J, Skandhan KP, Sahab Khan P, Sumangala B, Amith S. Estimation of calcium and magnesium in seminal plasma. A comparative study of calorimetry and Atomic Absorption spectrophotometry. Int J Analy BIO Analy Chem. 2013;3:23-6.
74. Shivaji S, Scheti K, Bharagava PM. Proteins in seminal plasma. Interface Sci. 1989.
75. Barfield JP, Yeung CH, Cooper TG. Characterization of potassium channels involved in volume regulation of human spermatozoa. Mol Hum Reprod. 2005;11(12):891-7. doi: 10.1093/molehr/gah208, PMID 16421215.
76. Mann T. The biochemistry of semen and of the male reproductive tract. London, Methuen and Company LTD, 1964.
77. Schmid TE, Grant PG, Marchetti F, Weldon RH, Eskenazi B, Wyrobek AJ. Elemental composition of human semen is associated with motility and genomic sperm defects among older men. Hum Reprod. 2013;28(1):274-82. doi: 10.1093/humrep/des321, PMID 23042799.
78. Breitbart H. Intracellular calcium regulation in sperm capacitation and acrosomal reaction. Mol Cell Endocrinol. 2002;187(1-2):139-44. doi:10.1016/S0303-7207(01)00704-3, PMID 11988321.
79. Yanagimachi R. Mechanism of fertilization in mammals. In:Jr.L.Mastroianni Biggers JD, editor. Fertilization and embryonic development in vitro. New York: Plenum press; 1981.
80. Lindemann CB, Goltz JS, Kanous KS. Regulation of activation state and flagellar wave form in epididymal rat sperm: evidence for the involvement of both Ca2+ and cAMP. Cell Motil Cytoskeleton. 1987;8(4):324-32. doi: 10.1002/cm.970080405, PMID 2826020.
81. Lornage J, Guerin JF, Czyba JC, Menezo Y. Influence of cations and albumin on human spermatozoa. Arch Androl. 1983;10(2):119-25. doi: 10.3109/01485018308987552, PMID 6860035.
82. Peterson RN, Freund M. Relationship between motility and the transport and binding of divalent cations to the plasma membrane of human spermatozoa. Fertil Steril. 1976;27(11):1301-7. doi: 10.1016/s0015-0282(16)42199-0, PMID 976503.
83. Marín-Briggiler CI, Vazquez-Levin MH, Gonzalez-Echeverría F, Blaquier JA, Tezón JG, Miranda PV. Strontium supports human sperm capacitation but not follicular fluid-induced acrosome reaction. Biol Reprod. 1999;61(3):673-80. doi: 10.1095/biolreprod61.3.673, PMID 10456844.
84. Pleban PA, Mei DS. Trace elements in human seminal plasma and spermatozoa. Clin Chim Acta. 1983;133(1):43-50.

doi: 10.1016/0009-8981(83)90019-0, PMID 6627676.
85. Dawson EB, Ritter S, Harris WA, Evans DR, Powell LC. Comparison of sperm viability with seminal plasma metal levels. Biol Trace Elem Res. 1998;64(1-3):215-9. doi: 10.1007/BF02783337, PMID 9845475.
86. Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, Paneth N, Wirth JJ. Cadmium, lead, and other metals in relation to semen quality: human evidence for molybdenum as a male reproductive toxicant. Environ Health Perspect. 2008;116(11):1473-9.
doi: 10.1289/ehp.11490, PMID 19057699.
87. Blein G, Bonsquet D, Clement M, Jean Y, Roberts KD, Chapdelaine A. Selenium in human semen. Fertil Steril. 1997;31:246-50.
88. Skandhan KP. Review on copper in male reproduction and contraception. Rev Fr Gynecol Obstet. 1992;87(12):594-8. PMID 1485076.
89. Skandhan KP, Valsa J, Sumangala B, Jaya V. Gold in semen: level in seminal plasma and spermatozoa of normal and infertile patients. Alex J Med. 2017; 53(1): 31-3. doi: 10.1016/j.ajme.2016.02.003.
90. Godatwar P, Prasad BS, Skandhan KP, Mehra BL, Singh G, Prakash J. Effect of Ayurvedic Medicine "Suvarna Bhasma" (Gold Ash) and "Atmagupta Churna" in normozoospermic infertile patients. Int J Sci Res. 2020.
91. Skandhan KP, Singh G, Prasad BS, Thakur AB, Godatwar PR, Rao N, Pandhya AR. Veracity of "Suvarna Bhasma" [Gold Ash], an indigenous ayurvedic preparation, as a therapeutic agent for male infertility. J Ayur. 2009; 3:83-6.
92. Riffo M, Leiva S, Astudillo J. Effect of zinc on human sperm motility and the acrosome reaction. Int J Androl. 1992; 15(3):229-37. doi: 10.1111/j.1365-2605.1992.tb01343.x, PMID 1399086.
93. Marmar JL, Katz S, Praiss DE, DeBenedictis TJ. Semen zinc levels in infertile and postvasectomy patients and patients with prostatitis. Fertil Steril. 1975;26(11):1057-63.
doi: 10.1016/s0015-0282(16)41470-6, PMID 1183629.
94. Skandhan KP, Mazumdar BN. Le zinc dans le plasma seminal (zinc in seminal fluid). Rev Franc Obstet Gynecol. 1985;80(12):891-8.