ASSESSMENT OF LEVEL OF POTASSIUM AND CALCIUM IN SEMEN AND THEIR EFFECT ON THE ACCOUNTS, SHAPES AND MOVEMENT OF SPERM

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Abstract. The study was designed to determine the effect of different levels of both elements (potassium and calcium) on some variables in the microscopic examination of semen (account and shape of sperms and their rate of movement within the semen plasma). In this study, 60 semen samples were collected from healthy people and divided into three groups based on the test results. Group (A) represented samples with positive results, and group (B) represented the average test results. At the same time, group (C) included weak results. The results were obtained after comparing the extent of the effect of calcium and potassium on the number, shape and movement of sperms between the three groups. The results showed that potassium had a significant effect on the number of sperm cells and the nature of movement in group (A) at the level of probability ($P \leq 0.05$). In group (B), the effect of potassium on the percentage of normal-shaped sperm cells was observed. In group (C), the effect of potassium level on the shape ratios of normal and abnormal cells. For calcium element, the study's results showed the effect of calcium element in group (A) on the percentage of motile cells, and the same effect of calcium applies to group (B). As for group (C), the effect of calcium was on the percentage of immobile cells and cells with an abnormal shape.

Keywords: semen, sperm, potassium, calcium.

Introduction

Semen is one of the fluids secreted by the typical male body. Its volume ranges between (1.5-5) milliliters during ejaculation, and this amount varies from one person to another. If a man has sexual intercourse twice a day, the amount of ejaculation in the second time will be less. In addition, the amount of ejaculation is affected by other factors, including testosterone levels or taking certain medications, such as in diabetics (Bassey *et al.*, 2019). Semen is produced in the testicles, which contain the seminiferous tubules that contain semen. Sertoli cells are responsible for producing semen. These cells settle in the basement membrane and stick together compactly, separated by a barrier of tight joints called the (blood-tests barrier), which prevents the entry of toxic substances into the tubules. Thus, it affects the components of the semen (Nail, 2019). Sertoli cell is mainly hormonally regulated by testosterone. Calcium is very important for the production of steroid hormones in the Leydig cells in the testicles, and calcium has an important role in the modulation of testosterone, which facilitates the development of gonadal cells as well as production of sperm (Morabbi & Karimian, 2024). Semen consists of a complex mixture that contains a variety of organic substances, which include proteins, fructose, and another group of inorganic substances that include mineral elements such as calcium, potassium, magnesium, zinc, and others, all of which are called nutrients and are necessary for continued fertility and reproduction. They are nutrients for sperm that swim in semen plasma (Bassey *et al.*, 2013; Marshall *et al.*, 2013; Piste Pravina *et al.*, 2013). Male fertility is a complex feature consisting of various physiological processes, including the growth and development of the reproductive system from birth to adolescence (Al-Azzawy, 2017). The process of sperm production begins at the age of 14 years and does not stop at all after that in an ordinary man, but it begins to slow down a little with age. The sperm that a man produces is always sufficient to obtain fertilization, and the rate of what one man produces reaches the average age of 60 million sperm in every single milliliter of semen, which has an average volume of about 2.75 milliliters in each ejaculation. This quantity decreases as men reach the age of (40) years. In addition to age, other factors can negatively affect the amount of semen. These include antibiotics, radiation, drugs and alcoholic beverages (Sansone *et al.*, 2018; Hamza *et al.*, 2012). The exposure to environmental pollutants and dietary intake in the areas of study and the level of macro and trace elements have crucial role in regulating of biological processes, including those linked to reproduction, among them are Ca and k. and the bioaccumulation of these elements in semen are more evident in semen than blood have noticeable effects on fertility (Nunzio *et al.*, 2022). It can be said that semen is responsible for fertilization and reproduction and if any defect occurs in its components, the man loses the ability to fertilize. Therefore, he will be subjected to a semen examination to investigate the presence of the problem or defect, and infertility constitutes a large part of the health problems in our society. There may also be many factors responsible for male infertility (Sansone *et al.*, 2018). This is why men unable to fertilize resort to semen testing (Bassey *et al.*, 2019). The examination includes collecting the sample through masturbation and keeping it in an incubator at a temperature ranging between 20-37 °C for a period of up to 30 minutes to dilute the sample. Sometimes, the sample does not dilute during this period, so the sample must be dealt with through mechanical mixing or digestion. Enzymatic or transferring the sample to a physiological medium helps the sample to liquefy, after which morphological tests begin by measuring the liquid's volume, color, and smell. Then, the pH is measured because the change in acidity in the semen makes the environment unsuitable for the sperm to live in. Then, it is subjected to microscopic examination and is determined through the number and shape of sperms. The sperm consists of three regions: head, neck and tail. When there are abnormalities in shape, such as two heads, a head connected to two tails, or a long tail, this indicates that the sperm's shape is abnormal and it cannot perform its role in the movement and fertilization of the egg.

The motor activity of the sperm is also measured, as it is either actively moving, slowly, or immobile. After that, the result is estimated (Bassey *et al.*, 2019; Wróblewska *et al.*, 2015). When the result is negative, there are two possibilities. Either the problem is organic, leading to weak sperm production, such as a disease in the testicles or a defect in the secretion of the prostate gland, or the reason may be due to the lack of semen plasma contents of proteins, sugars, vitamins such as vitamin E, or mineral elements. (such as calcium and potassium). Calcium is essential for muscle contraction, egg activation, building strong bone2013s and teeth, blood clotting and nerve propulsion (Piste Pravina *et al.*, 2013; Tvrdá *et al.*, 2013; Bassey *et al.*, 2013), transmitting and regulating heartbeats through its role in regulating the electrical activity of the heart, as calcium molecules are bound inside cells in the heart muscle. The presence of calcium receptor, vitamin D receptor, and calcium channels in the testes, male reproductive tract and human sperms, suggests that calcium and vitamin D and may modulate the function of male reproductive system (Yahyavi *et al.*, 2023) As for potassium, it is found inside cells and plays an essential role in vital processes (Valsa *et al.*, 2015; Bassey *et al.*, 2013). Potassium and calcium are mineral elements found in semen plasma. Therefore, the study was designed in our research to determine the extent of the effect of potassium and calcium levels on the number and shape of sperms, in addition to the nature of sperms movement (EFSA *et al.*, 2016).

Materials and Methods

Samples used

In this study, samples consisting of 60 semen samples, randomly selected from healthy people, were collected after a period of abstinence from masturbation for 72 hours. The samples were collected in two private diagnostic laboratories in Mosul from 10/6/2019 to 1/8/2020 (Iraq). Ages ranged from (20-45) years, and were placed in sterile containers for collecting semen samples. Then, the samples were placed in the incubator at 37 degrees Celsius for 30 minutes. After that, samples were divided into three groups based on the results of laboratory semen tests.

Group (A) included samples from people with positive results regarding number, shape, and sperm motility.

Group (B) included samples from people whose results were average regarding number, shape, and sperm motility.

Group (C) included samples from people with poor results regarding number, shape, and sperm motility.

Laboratory tests

1. Microscopic examination

Through it, the number of sperm within the sample, the shape of the sperm, and the nature of its movement were estimated using an Olympus microscope from Japan (Björndahl *et al.*, 2010).

2. Biochemical examination

2.1. Estimation of calcium in semen samples

Calcium level was estimated using the enzymatic method (Biolabo), France, and a spectrometer with a wavelength of 570 nm (Choi *et al.*, 2014).

2.2. Estimation of potassium in semen samples

Potassium level in semen was estimated using a Reflotron strip (Roche, Hitachi), Japan (Sağlam *et al.*, 2015).

Statistical analysis

The results were analyzed statistically for all groups using ANOVA ONE-WAY for Person, and the results were tested probabilistically at the level ($P \leq 0.05$) (Luay & Tareq, 2010).

The consent of the donors in the three groups was obtained before starting the study, and it was explained to them how important this study was in improving the health situation of affected people in the future.

Ethical statement

1. This material is the authors' own original work, which has not been previously published elsewhere.
2. The paper reflects the authors' own research and analysis in a truthful and complete manner.
3. The paper properly credits the meaningful contributions of co-authors and co-researchers.
4. The results are appropriately placed in the context of prior and existing research.
5. All sources used are properly disclosed (correct citation). Literally copying of text must be indicated as such by using quotation marks and giving proper reference.
6. All authors have been personally and actively involved in substantial work leading to the paper, and will take public responsibility for its content.

After taking Semen samples from the patients, they were informed and we obtained their consent to conduct this research.

Results

Table 1 shows the measurement of the correlation coefficient (R) between the level of potassium and calcium with the variables in the semen examination of the group (A) at probability ($R < 0.3$). The Table shows a moderate direct relationship between potassium and the number of sperm. In contrast, the relationship was weak. Table 2 indicates that potassium level has a moderate positive correlation with the proportions of normal-shaped sperm cells and an inverse correlation with the proportions of abnormal cells. The table also shows a weak inverse correlation with non-motile and poorly motile cell proportions. The correlation coefficient for calcium in group (B) is a solid direct correlation with the percentage of motile cells and a strong inverse correlation with the percentage of non-motile cells. Both potassium and calcium recorded a weak positive correlation and a weak inverse correlation, respectively, with the number of sperm cells. Table 3 shows that there is a moderate inverse correlation between potassium and the percentage of abnormally shaped sperm cells and also a moderate inverse correlation with the percentage of non-motile sperm. As for the correlation of calcium with the number, shape, and movement of a group (C), the sperm was weak, relative, direct, and inverse. Table 4 also shows a comparison between groups (A, B, and C) in the level of potassium and calcium, number of sperm cells, proportions and shapes of normal and abnormal sperm, as well as the rates of sperm motility between (motile, weakly motility, and

Table 1

Correlation coefficient of potassium and calcium level with variables in semen group (A)

A	Number	Normal	Abnormal	Motile	Sluggish	Non-motile
Potassium	0.57	0.29	-0.29	0.57	0.30	-o.53
Calcium	0.00	0.29	-0.29	0.40	0.09	-0.31

Table 2

Correlation coefficient (R) between potassium and calcium levels with semen variables for group (B)

B	Number	Normal	Abnormal	Motile	Sluggish	Non-motile
Potassium	0.01	0.59	-0.59	0.43	-0.08	-o.27
Calcium	-0.06	0.07	-0.07	0.68	0.36	-0.61

Table 3

Correlation coefficient (R) between potassium and calcium levels with semen variables for group (C)

C	Number	Normal	Abnormal	Motile	Sluggish	Non-motile
Potassium	0.05	-0.13	-0.54	0.24	-0.12	-0.54
Calcium	0.20	0.01	-0.16	0.26	0.19	-0.21

Table 4

Comparison of semen variables between groups (A, B, C)

P-value	Examination of semen	
$*2.21*10^{-13}$	sperm count	1
$*1.56*10^{-15}$	shapes of normal sperms	2
0,0016	shapes of abnormal sperms	3
$*4.36*10^{-12}$	rates of motile sperm	4
0,0214	rates of weak motile sperms	5
0,0216	rates of non-motile sperm	6

Note: * Indicates a significant difference with a probability value (P-value) of less than 0.05

non-mobile). When comparing the three groups for semen examination, there are significant differences in number of sperm, percentage of normal-shaped sperm cells, and percentage of motile sperm cells. Table 5 shows a significant difference in the probability value of less than 0.05 in the potassium and calcium level between groups (A, B, and C). This difference between groups may be the reason for the difference in semen variables, including the number and shape of the movement of sperm cells, which is what the study aimed for.

Discussion

The percentage of normal-shaped sperm, an inverse relationship with the percentage of abnormally shaped cells, a moderate positive relationship with the percentage of motile cells, and a strong inverse relationship with the percentage of non-motile cells. As for the

Table 5

The comparison of the level of potassium and calcium in groups (A, B, C)

P-value	Elements concentration
$6.71*10^{-9*}$	Potassium
0,000135585	Calcium

Note: * Indicates a significant difference with a probability value (P-value) of less than 0.05

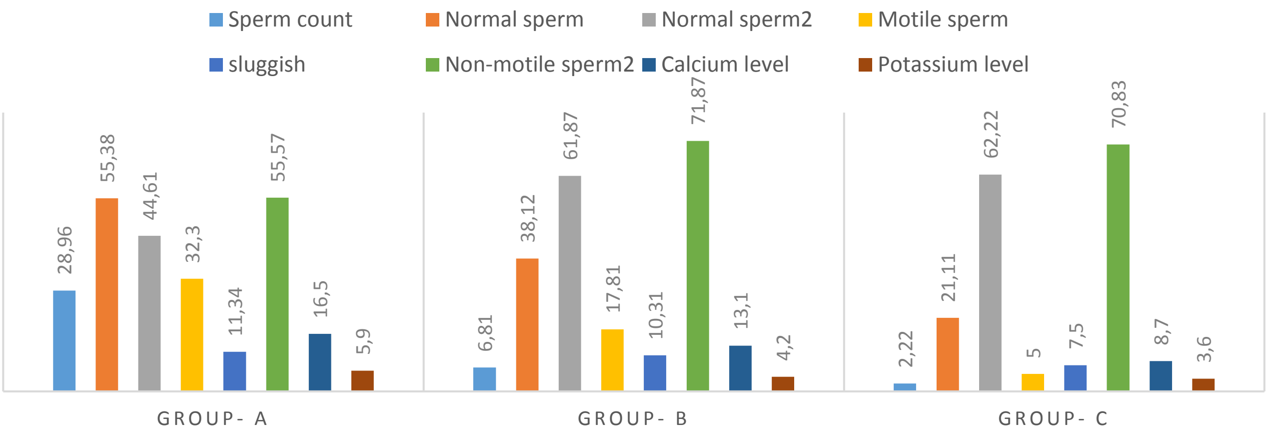

Fig. 1. The comparison of the levels of potassium and calcium, the number of sperm cells, and the proportions of cell shapes and movement between the three groups (A, B, C)

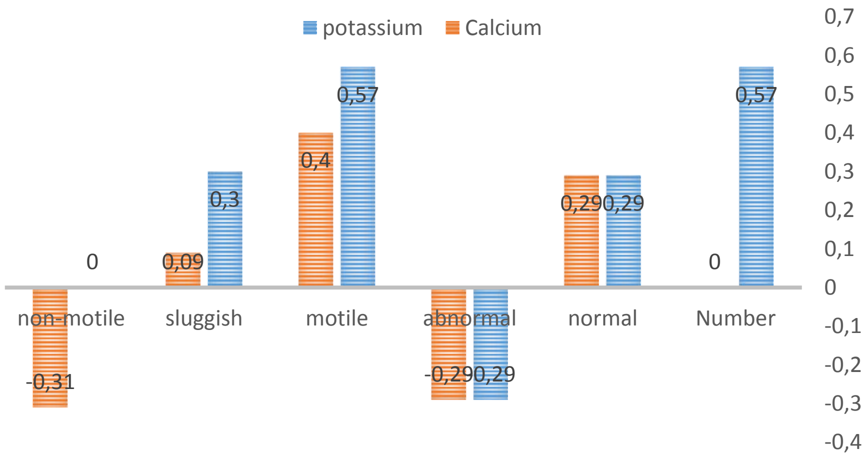

Fig. 2. Correlation coefficient of potassium and calcium level with variables in semen group (A)

relationship of potassium level with the percentage of weakly mobile cells, its relationship is almost direct and relatively weak.

Figure 2 shows the correlation coefficient between the level of calcium and sperm in the semen plasma of group (A); it indicated that there is no correlation with the number of sperm cells and that there is a weak direct correlation with the shape of normal cells and a weak inverse correlation as well with the percentage of sperm with abnormal shapes. The correlation

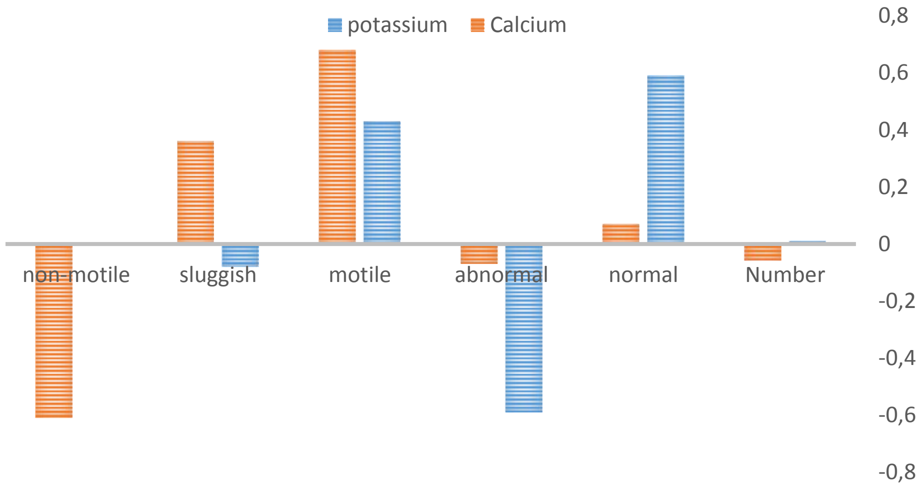

Fig. 3. Correlation coefficient (R) between potassium and calcium levels with semen variables for group (B)

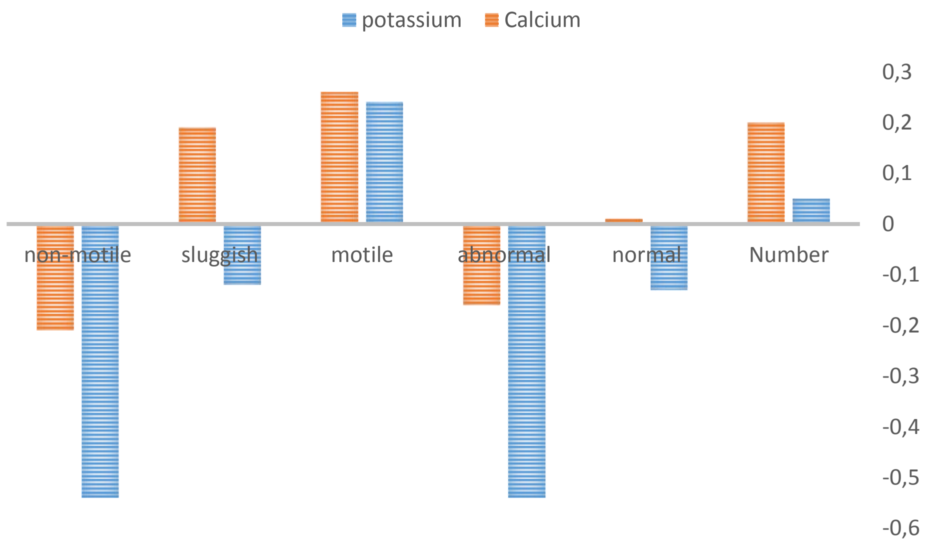

Fig. 4. Correlation coefficient (R) between potassium and calcium levels with semen variables for group (C)

of calcium with the proportions of motile cells had a moderate positive correlation, and there was no correlation with cells with weak movement, while there was a weak inverse correlation with non-motile cells.

From the above (Fig. 3, 4), it can be concluded that low levels of potassium and calcium levels in semen plasma may affect sperm, as a group (A) was characterized by a higher number of sperm cells compared to groups (B and C), with a significant difference, and this is consistent with Clarke, 2017; Valsa *et al.*, 2015; Choi *et al.*, 2014. The role of potassium inside cells in protein synthesis and its necessary presence in the development of oxidative stress helps keep cells alive. It is similar to group (B) compared to group (C). Also, the role of potassium and calcium in maintaining the sperm cells in a standard shape through which they can perform physiological functions and reduce the percentage of abnormal cells with a significant difference between the three groups, as indicated by our study and confirmed by Scott *et al.*, 2016;

Al-Azzawy, 2017; Sutanto & Heijman, 2019, the presence of potassium inside the sperm more than in the plasma fluid. Sperm maintains osmotic pressure and thus maintains the cells' standard shape. No significant direct effect of calcium was observed on the percentage of sperm cells in terms of shape, which is consistent with Kimsakulvech *et al.*, 2016; Banjoko & Adeseolu, 2013; Tvrdá *et al.*, 2013. The study by Bootman & Bultynck, 2020 confirmed that increasing intracellular calcium will lead to structural modifications of the cell and activate the role of phospholipase, which affects the cell's plasma membrane. Thus, morphological deformities occur. In group (A), potassium and calcium directly affected the percentage of motile cells, as indicated by (Sutanto & Heijman,2019, Scott *et al.*, 2016, WHO, 2010). The entry of calcium into specific channels helps the movement of the tail in the sperm. It also plays a significant role in the activity of the sperm inside the oviduct and thus increases the chances of fertilization. Potassium plays a role in maintaining the activity of sperm cells by maintaining osmotic pressure. In group (B), the nature of sperm cell motility was better than in group (C). As for the percentages of non-motile cells (Fig. 1), there was a non-significant difference (A) compared to groups (B and C), and this also applies to the percentages of cells with weak movement. (Nunzio *et al.*, 2022).

Conclusion

More studies are needed to find out the threshold values for male infertility and sperm dysfunction, and the determination of the possibly hazardous elements, in human semen and blood serum simultaneously, can be of value to determine some relations with environmental pollution and dietary intakes.

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Conflict of interest: the authors declare that there is no conflict of interest.

References

AL-AZZAWY L.H.A. (2017): The Impact of Cigarette Smoking on Levels of Sex Hormones and Zinc in Blood of Smokers. *Ibn AL-Haitham Journal For Pure and Applied Science* **24**(2).

BANJOKO S.O. & ADESEOLU F.O. (2013): Seminal plasma pH, inorganic phosphate, total and ionized calcium concentrations in the assessment of human spermatozoa function. *Journal of clinical and diagnostic research: JCDR* **7**(11), 2483.

BASSEY I.E., ESSIEN O.E., UDOH A.E., IMO I.U. & EFFIONG I.O. (2013): Seminal Plasma Selenium, Calcium, Magnesium and Zinc Levels in Infertile Men. *Journal of Medical Sciences* **13**(6), 483-487.

BASSEY I.E., ISONG I.K.P., ESIERE K.U.S., ESSIEN O.E., UDOH A.E. & AKPAN U.O. (2019): Seminal Oxidative Stress Markers, Calcium, Magnesium, and Semen Profile of Infertile Diabetic and Nondiabetic Nigerian Men. *J Appl Basic Med Res* **9**(3), 159–164.

BJÖRNDAHL L., MORTIMER D., BARRATT C.L.R., CASTILLA J.A., MENKVELD R., KVIST U., ALVAREZ J.G. & HAUGEN T.B. (2010): Basic semen analysis. In: *A Practical Guide to Basic Laboratory Andrology*, pp. 33 – 76. Cambridge: Cambridge University Press.

BOOTMAN M.D. & BULTYNCK G. (2020): Fundamentals of cellular calcium signaling: a primer. *Cold Spring Harbor perspectives in biology* **12**(1), a038802.

CLARKE K. (2017): Semen was used to make invisible ink, in news you could never make up. *CBC Life.* https://www.cbc.ca/life/wellness/five-surprising-facts-about-semen-from-a-men-s-health-expert-1.4082839.

CHOI E., LYU J., PARK J. & KIM H.Y. (2014): Statistical methods used in articles published by the Journal of Periodontal and Implant Science. *Journal of periodontal & implant science* **44**(6), 288–292.

EFSA PANEL ON DIETETIC PRODUCTS, NUTRITION AND ALLERGIES (NDA), TURCK D., BRESSON J.L., BURLINGAME B., DEAN T., FAIRWEATHER-TAIT S. ... & NASKA A. (2016): Dietary reference values for potassium. *EFSA Journal* **14**(10), e04592.

HAMZA A.A., SULAYMAN S.D. & HAMZA M.A. (2012): Determination of fructose and some mineral elements concentration in the seminal plasma of rams. Journal of Education and Science **25**(3), 69–77.

KIMSAKULVECH S., SUTTIYOTIN P. & PINYOPUMMIN A. (2016): Onset and duration of ejaculatory suppression effect of tamsulosin in goat. *Agriculture and Natural Resources* **50**(4), 306–309.

LUAY A.-H. & TAREQ A. (2010): Biochemistry. Ibn Al-Atheer House, University of Al Mosul.

MARSHALL W.J., LAPSLEY M., DAY A. & AYLING R. (2014): *Clinical Biochemistry: Metabolic and Clinical Aspects.* Churchill Livingstone, 996 pp.

MARZEC-WRÓBLEWSKA U., KAMIŃSKI P., ŁAKOTA P., LUDWIKOWSKI G., SZYMAŃSKI M., WASILOW K. ... & JERZAK L. (2015): Determination of rare earth elements in human sperm and association with semen quality. *Archives of environmental contamination and toxicology* **69**, 191–201.

MORABBI A. & KARIMIAN M. (2024): Trace and essential elements as vital components to improve the performance of the male reproductive system: implications in cell signaling pathways. *Journal of Trace Elements in Medicine and Biology* **83**, 127403.

NAIL R. (2019): Normal sperm count: Everything you need to know. *Medical news today*. https://www.medicalnewstoday.com/articles/324821.

NUNZIO A.D., GIARRA A., TOSCANESI M., AMORESANO A., PISCOPO M., CERETTI E. ... & MONTANO L. (2022): Comparison between macro and trace element concentrations in human semen and blood serum in highly polluted areas in Italy. *International Journal of Environmental Research and Public Health* **19**(18), 11635.

PISTE PRAVINA P.P., DIDWAGH SAYAJI D.S. & MOKASHI AVINASH M.A. (2013): Calcium and its Role in Human Body.

SAĞLAM H.S., ALTUNDAĞ H., ATIK Y.T., DÜNDAR M.Ş. & ADSAN Ö. (2015): Trace elements levels in the serum, urine, and semen of patients with infertility. *Turkish journal of medical sciences* **45**(2), 443–448.

SANSONE A., DI DATO C., DE ANGELIS C., MENAFRA D., POZZA C., PIVONELLO R. ... & GIANFRILLI D. (2018): Smoke, alcohol and drug addiction and male fertility. *Reproductive biology and endocrinology* **16**(1), 1–11.

SCOTT I., RAMIREZ-REVECO A. & PARODI J. (2016): The restraint of bovine sperm cell motility increases survival: role of extracellular calcium in the phenomena. *J Vet Sci Technol* **7**(359), 2.

SUTANTO H. & HEIJMAN J. (2019): The role of calcium in the human heart: with great power comes great responsibility. *Front Young Minds* **7**(65), 10-3389.

TVRDÁ E., LUKÁČ N., SCHNEIDGENOVÁ M., LUKÁČOVÁ J., SZABÓ C., GOC Z. ... & MASSÁNYI P. (2013): Impact of seminal chemical elements on the oxidative balance in bovine seminal plasma and spermatozoa. *Journal of veterinary medicine*.

TVRDÁ E., SIKELI P., LUKÁ J., MASSÁ P. & LUKÁ N. (2013): Mineral nutrients and male fertility. *Journal of microbiology, biotechnology and food sciences* **3**(1), 1–14.

VALSA J., SKANDHAN K.P., KHAN P.S., AVNI K.P.S., AMITH S. & GONDALIA M. (2015): Calcium and magnesium in male reproductive system and in its secretion. I. Level in normal human semen, seminal plasma and spermatozoa. *Urologia Journal* **82**(3), 174–178.

WHO (2010): WHO laboratory manual for examining and processing human semen. Fifth edition. Switzerland, 286 pp.

YAHYAVI S.K., BOISEN I.M., CUI Z., JORSAL M.J., KOOIJ I., HOLT R. ... & JENSEN M.B. (2023): Calcium and vitamin D homoeostasis in male fertility. *Proceedings of the Nutrition Society* 1–14.