DISTRIBUTION OF TRACE ELEMENTS IN PLACENTA AND AMNION OF WOMEN FROM UPPER SILESIAN REGION OF POLAND

Suprewicz, K.[1], Kozikowska, I.[1], Stawarz, R.[1], Sławska, H. [2], Piekarz, A.[3]

[1]*Department of Zoology of Vertebrates and Human Biology, Pedagogical University of Cracow, Poland*
[2]*Department of Gynecology and Obstetrics, Neonatal Intensive Care Unit, Bytom, Medical University of Silesia, Poland*
[3]*Department of Gynecology and Obstetrics, Medical University of Silesia, Katowice, Poland*

ABSTRACT

The levels of trace elements were examined in human placenta and amnion taken from 40 women living in Upper Silesian Region in Southern Poland. Placental and amnion concentrations of zinc, iron, copper were determined by Atomic Absorption Spectrometry (AAS). All trace elements were detected both in placenta and amnion. Placenta can play an important role in biomoniotoring of metals in environment. The distribution of investigated metals was diversified in different parts of the placenta as expressed by different levels of selected metals. The highest concentration was observed in case of iron content in central part of placenta. The higher level is probably the effect of the high demand of the organism for this metal. It was also observed that accumulation Fe, Zn and Cu in different parts of placenta correlates with age of mothers. The concentration of iron increases along with age. Medium positive correlations were found between Fe and Cu (p=0,001), and weakly positive correlation between Zn and Cu (p=0,01) were found. Correlation between zinc and cooper, as well as iron and copper can be an effect of high demand for these elements by women's metabolism for adequate fetus development.

Key words: human placenta, amnion, zinc, copper, iron

INTRODUCTION

Prenatal period is a time when embryos entirely depend on mother organism. Firstly, harmful substances are eliminated in diffusion process by growing embryo but in the further stage where more efficient mechanism is needed, this role is complemented by placenta, which is transitional fetal organ. Placenta creates connection between mother and child’s circulation system which occur in mammals.

Fetal circulation consists of two systems: the fetal and mother's part (Bartel, 2002). Both of them are divided by thin tissue layer called placental barrier, which allows oxygen and nutrients to transport from mother to children (Moore, 2003). Except for necessary substances for proper grow of fetus, also undesirable substances such medicines, stimulants can pass through placental barrier (Sadler, 1993). Pregnant mothers are exposed to a wide variety of foreign chemicals. Placenta is being functional organ right after third month of pregnancy. Among microelements which are necessary for a lot of metabolic processes occurring in the organism, are copper, zinc and iron. High or low level of these trace elements can cause many disorders and disfunctions.

Zinc plays very important role in the well-functioning biological system by stabilizing work of many enzymes and plays role in the activity and protein synthesis or nucleic acids (Sky Peck, 1986). Copper is essential component for many enzymes (Gibson, 1989; Danks, 1988) and catalyze a lot of biochemical processes (Pasternak, 1995). Deficiency of zinc and copper is very unfavorable during pregnancy. These metals can contribute in developing of incorrect tissues and organs structure, they are able to change protein metabolism or nucleic acids and also disturb correct fetus growing (Ashworth and Antipatis, 2001; Keen and others, 2003). As a result early fetal deaths, defects of respiratory, circulatory or bone systems can occur (Fields and others, 1990; Keen and others, 1998).

High amount of iron is toxic and therefore there are specific mechanisms which are responsible for keeping this component on correct level. Although taking iron during pregnancy is very high, in most cases this level is still not sufficient for fetus demands (Bard, 1994). Because of this, it was noticed in a lot of cases that pregnant women had anemia. Anemia during pregnancy presents danger also for growing fetus. Lack of iron during pregnancy can increase blood pressure (Crowe and others, 1995), decrease of functionality and brain efficiency (Kwik-Uribe and others, 2000; Rao and Jagadeesan, 1996; Rao and others, 1999; Soewindo, 1995; Walters and others, 1973).

The main objective of this study was to investigate medium content of iron, zinc and copper in various parts of placenta and amnion in three age groups of women. It seems to be interesting to estimate differences in amount of Fe, Cu and Zn in particular parts of placenta and research in which part of placenta is the highest level of this metals.

MATERIALS AND METHODS

Amnion and two parts of placenta (central placenta, marginal placenta) were collected and frozen after delivery from the Clinic of Gynecology and Obstetrics (Medical University of Upper Silesian, Katowice). Tissue was obtained from 40 patients. We examined a total of 120 samples. The age of mothers ranged from 18-40 years.

A few grams of tissue samples were mineralized in laboratory after dray mass of each sample had been obtained through drying. All dry material of each sample was placed in separate mineralization tubes and mineralized by adding 2 mL HNO_3-$HClO_4$ (4:1) mixture and heating at 120°C. The resulting solution was diluted to 10 mL with demineralizated water. Metal contents (Cu, Zn, Fe), were determined by the voltametric method (ASV).

Detailed data about women in a patient survey about diet and lifestyle was collected. An analysis takes into account preferred lifestyle drugs (alcohol, tobacco, coffee), used drugs both before and during pregnancy, contact with chemicals. There was also the basic information about the status of the child after birth. The measurement results are expressed in ($mg \cdot kg^{-1}$d.m.). The data obtained were analyzed by Statistica 9.0.

RESULTS

The examined metals were found in all tissues. The results obtained were analyzed statistically.

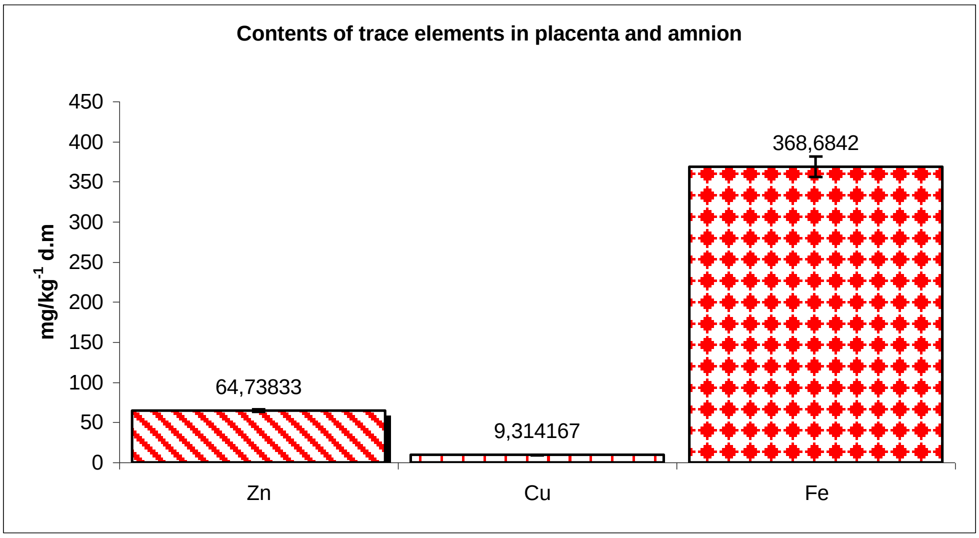

Figure 1 The average content of elements studied in the placenta and amnion

($mg \cdot kg^{-1}$ d.m± Std.Err)

Figure 1 illustrates average level of metals: zinc, copper and iron. Comparing the analyzed metals, it is noticed that the content of iron in analyzed tissues is the highest (368.682 $mg \cdot kg^{-1}$ s.m). Among the metals content in the placenta and amnion, the lowest level of copper was recorded (9.314 $mg \cdot kg^{-1}$ d.m.).

The distribution of investigated elements in various part of placenta and amnion was checked with Shapiro-Wilk test for normality at $p<0.05$. A Kruskala – Wallis nonparametric test was used to determine differences among experimental groups in concentration of trace elements. Correlations between Zn, Cu and Fe were calculated by Spearman test.

Table 1 Concentration of copper in various part of placenta and amnion for specific women age range (mg·kg^{-1}d.m)

Part of placenta Age range	I-amnion (mg·kg^{-1} d.m±Std.Err.)	II-marginal placenta (mg·kg^{-1} d.m±Std.Err.)	III-central placenta (mg·kg^{-1} d.m±Std.Err.)
18-24	10.200±1.258	8.640±2.201	6.980±0.954
25-34	13.604±1.110	7.693±0.322	7.111±0.253
35-40	13.317±2.549	7.467±0.783	6.433±0.658

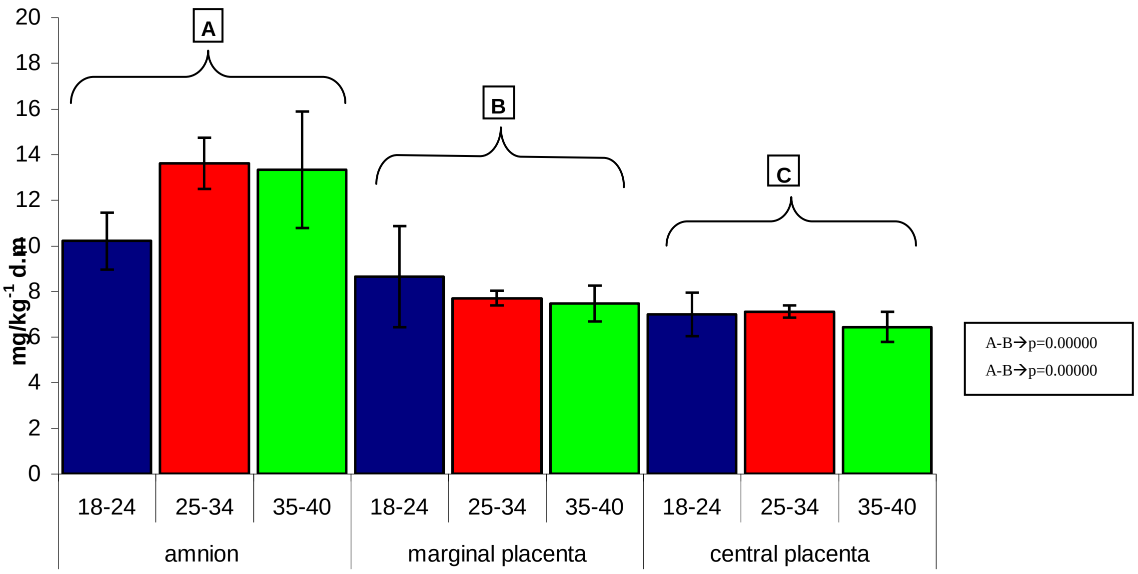

Figure 2 Distribution of copper within the placenta for specific women age range (mg·kg^{-1} d.m± Std.Err); I-amnion, II-marginal placenta, III-central placenta.

Average contents of copper in different parts of placenta and amnion of three age groups of women are presented in the Figure 2 and Table 1. On a basis of results it is noticed, that Cu contents for different age groups of women in placenta and amnion is diverse. The highest content of this element was found in the first age group in the amnion (10.200±1.258mg·kg^{-1} d.m.). The lowest level of copper was observed-in the first group age of women (18-24 years old) in central part of placenta (6.980±0.954 mg·kg^{-1}d.m).

Within theage group of women there were found statistically insignificant differences in the average content of copper (p=0.566), statistically insignificant differences in various parts of placenta and amnion within the first group age of women (p=0.151). Statistically significant difference in the average cooper content were found in different parts of the placenta and amnion within the second (p=0.000) and third (p=0.050) age group of women.

Table 2 Concentration of iron in various part of placenta and amnion for specific women age range (mg·kg^{-1} d.m)

Part of placenta Age range	I-amnion (mg·kg^{-1} d.m±Std.Err.)	II-marginal placenta (mg·kg^{-1} d.m±Std.Err.)	III-central placenta (mg·kg^{-1} d.m±Std.Err.)
18-24	280.100±38.455	401.480±66.259	457.440±63.080
25-34	238.289±18.077	400.525±16.087	433.107±20.887
35-40	200.083±30.186	476.633±56.144	487.700±48.909

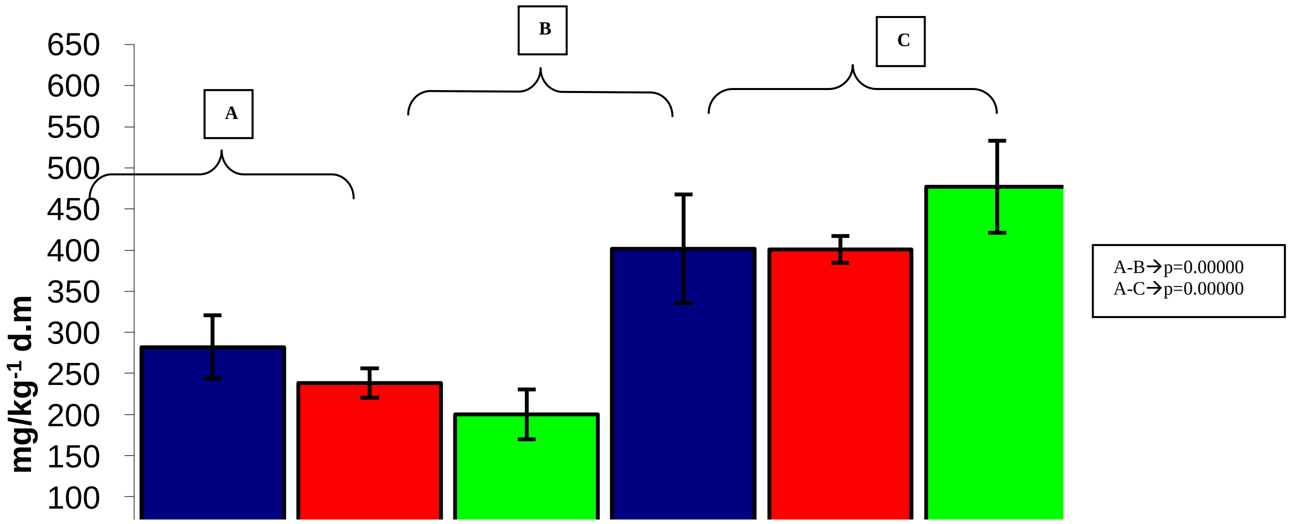

Figure 3 Distribution of iron within the placenta for specific women age range (mg·kg^{-1} d.m± Std.Err); I-amnion, II-marginal placenta, III-central placenta

The highest content of this element was found within the third age group (35-40 years old) in the cental part of placenta (487.700±48.909 mg·kg^{-1}d.m.). In this age group of women it was similar content of iron in the central and marginal parts of placenta.

The lowest level of Fe was noticed in the fetal membrane, where the average amount of this metal reached 239.49 mg·kg^{-1} d.m. In this tissue, the lowest content of metal was found in the third age group (35-40 years old).

Average contents of iron in different parts placenta and amnion of three age groups of woman are presented in the Figure 3 and Table 2.Within the age group of women there were found statistically insignificant differences in the average iron content (p=0.796), statistically insignificant differences in different parts of the placenta and amnion within the first group age of women (p=0.101). Statistically significant difference in the average iron content were found in different parts of the placenta and amnion within the second (p=0.000) and third (p=0.005) age group of women

Table 3 Concentration of zinc in various part of placenta and amnion for specific women age range ($mg \cdot kg^{-1}$ d.m)

Part of placenta Age range	I-amnion ($mg \cdot kg^{-1}$ d.m±Std.Err.)	II-marginal placenta ($mg \cdot kg^{-1}$d.m±Std.Err.)	III-central placenta ($mg \cdot kg^{-1}$ d.m±Std.Err.)
18-24	84.800±5.841	72.300±8.795	78.480±3.511
25-34	61.214±3.234	59.975±2.218	65.711±2.601
35-40	65.350±4.162	60.067±3.478	60.633±2.949

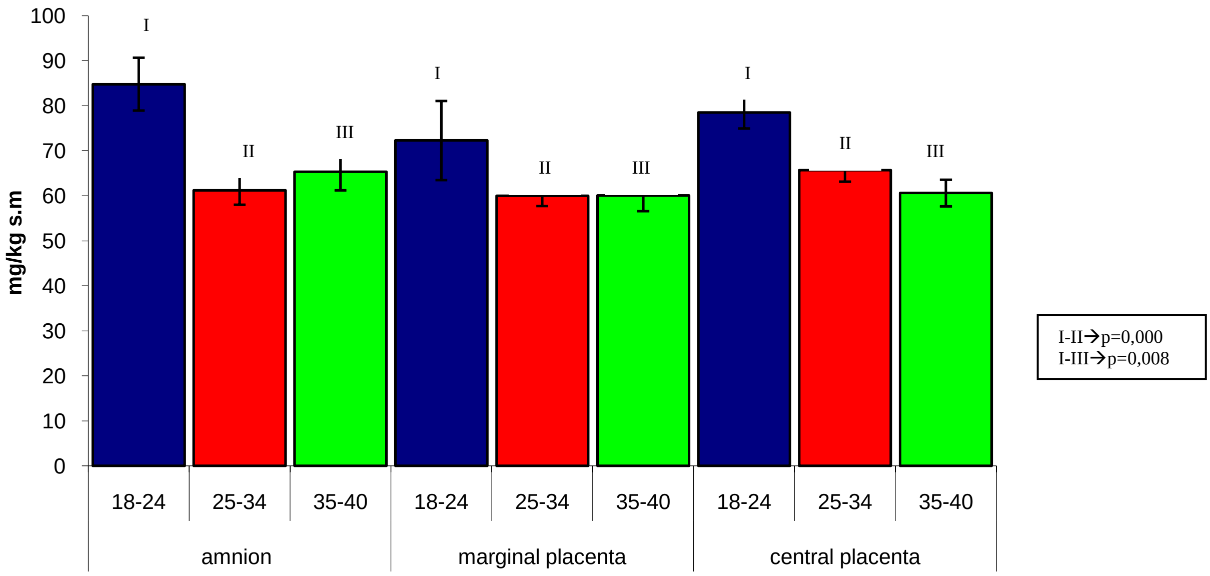

Figure 4 Distribution of zinc within placenta for specific women age range ($mg \cdot kg^{-1}$d.m±Std.Err); I-amnion, II-marginal placenta, III-central placenta

Average contents of zinc in different parts placenta and amnion in three age groups of women are presented in the Figure 4 and Table 3. In all examined tissues there were found large quantities of this element. The highest content of this element was found in the first age group in the marginal part of placenta (78.53 $mg \cdot kg^{-1}$ d.m). The lowest amount of zinc was found in the marginal part of the placenta of women of a research group 25-34 years (59.975±2.218 $mg \cdot kg^{-1}$ d.m).

Within the age group of women there were found statistically significant differences in the average zinc content (p=0,000), and statistically insignificant differences in different parts of the placenta and amnion.

We calculated correlations between investigated metals (Cu, Zn and Fe). The results are presented in Table 4. Positive correlation Zn/Cu (R=0.151), and medium positive correlations Cu/Fe (R=0.365) correlations in the examined parts of the placenta and amnion were found negligible.

Table 4 Spearman correlation coefficients between trace element in various parts of placenta and amnion from women

	Cu	Fe
Zn	p=0.010 R=0.151	p=0.869 R=0.015
Cu		p=0.000 R=0.365

With the increase of Zn in tissue of placenta and amnion increases the level of Cu and with the increase of Cu also Fe level is higher.

DISCUSSION AND CONCLUSION

Sorell and Graziano (1990) carried out research on interactions between metals in human organism. They noticed that decrease of transporting elements such as Zn, Fe or Cu from placenta to fetus occurs just at low level of cadmium. On the other hand, concentration of copper in the placenta tends to positive correlation with the age of mother that does not have any addictions (Kantola et al., 2000). Other scientists reported on the existence of positive correlation between cadmium and zinc and they also claim that smoking causes decrease of zinc value in the placenta. It shows that smoking has big influence on zinc level in the placenta of pregnant women (Korpela et al., 1986; Kuhnert et al., 1993). According to Swanson and King (1987), different behaviours of both copper and zinc in the placenta, causes that together with increasing level of cadmium in placenta also the level of copper and zinc in placenta changes in the ratio for the better copper.

According the researches which were conducted in Katowice, it was shown that Zn level depends on women's age. The content of this metal decreases with mother's age, what was confirmed by Richter et al. (1999) research. Kantola et al. (2000) have found dependence between smoking and zinc transport by placental barrier. They prove that concentration of both zinc and copper in the mother's blood and mother's placenta have much in common with environment pollution, smoking, pregnancy period, amounts of births and mother's age. Magnesium and calcium limit zinc absorption, what can cause serious defects of organism functions (in the bone or reproductive systems) (Kabata-Pendias, Pendias, 1993). Research in

Silesia has shown that with zinc level increase in placenta, also copper level increases. Together with an increase of copper level in the placenta, iron also increases.

Basic function of copper in the organism is to take part into oxidation-reduction processes, where it exists as coenzyme. Copper controls metabolism as well as iron and collagen transport. Copper excess decreases hemoglobin concentration and damages liver and kidneys (Steńczuk, 1990). Placenta has blocked transport of copper from mother into fetus direction (Krachler at al, 1999; Rossipal, 2000). General content of Cu in the placentas in 1975-2000 is oscilating under 0.59±1.18 $\mu g.g^{-1}$ w.m limit, with average value 0.9 $\mu g.g^{-1}$ w.m (Iyengar and Rapp, 2001).

According to researches which were carried out within last several years, we can observe huge variety of copper content in the human placenta. Maximum value were observed in the 1978, it was 7.06±1.04 $\mu g.g^{-1}$ d.m (Riemschneider and Martis, 1978) and the lowest value was observed in the 1988 (4.40±0.70 $\mu g.g^{-1}$ d.m). Researches carried out in the Silesia area proved that the value of copper in the placenta is on the really high level (9.314±4.459 $mg.kg^{-1}$ d.m). Investigation shows that distribution of this metal depends on mother's age and specific part of placenta and amnion tested. Older women concentrate higher amount of copper and distribution of this element in the placenta decreases in the direction: amnion - marginal placenta - central placenta. It proves that not whole amount of absorbed metal is transported with the same frequency by individual parts of placenta. On the border of investigated parts of placenta there should exist some mechanism, which would prevent from total transport of copper.

General content iron in placenta between 1975 and 2000 varies in the range of 43-115 $\mu g.g^{-1}$ w.m with average value of 69 $\mu g.g^{-1}$ w.m (Iyengar and Rapp, 2001). The data collected in Katowice demonstrates a positive correlation ($p = 0.000$) between various part of placenta and amnion, what is combined with various distribution of metal within organ. Together with woman age it was observed higher value of iron in the placenta. We can conclude that experienced women are aware of results of this element deficiency, and they try to supplement it. Researches from 1996 conducted by Sriramachari et al.(1996) states that value of iron reached 256±94 $\mu g.g^{-1}$ d.m. Other results obtained by Canteo et al. (1996), demonstrates higher Fe level (326±198 $\mu g.g^{-1}$ d.m). Similar results as Canteo et al. were observed in 2009 in Katowice. Researches in Silesia show that value of iron is about 368.684±137.922 $mg \cdot kg^{-1}$ d.m. These results present increasing level of iron in the placenta within the time of several years.

Increase of environment pollution by anthropogenic and socio-economic activities, has very unfavourable influence on human health and indirectly affects growing fetus, which depending on how toxic metals are, can be less or more threatened (Haworth at al, 1980; Wier at al, 1990; Cliver at al, 1995).

REFERENCES

1. ASHWORTH, C.J., ANTIPATIS, C. 2001. Micronutriens programming of development throughout gestation. In *Reproduction*, vol. 122, 2001, p. 527-535.
2. BARD, J.L. 1994. Iron deficiency: assessment durning pregnancy and its importance in pregnant adolescents. In *American Journal of Clinical Nutrition*, vol. 59, 1994, p. 502-508.
3. BARTEL, H. 2002. *Embriologia*. Warszawa : Wydanie Lekarskie PZWL, 2002, p. 133-146.
4. CANTEO, J.A., PASTANER, J.P., NIEVES, S., RAMOS, M., MULLICK, F.G., KALER, S.G. 1996. The assessment of trace elements and toxic metal levels in human placental tissues. In *Metal Ions in Biology and Medicine*, Ed. Ph. Collery, vol. 4, 1996, p. 522-524.
5. CLIVER, S.P., IINNI, I., 1995. The effect of cigarette smoking on neonatal anthropometrics measurements. In *Obstetrics and Gynecology*, vol. 85, 1995, p. 625-630.
6. CROWE, C., DANDEKAR, P., FOX, M., DHINGRA, K., BENNET, L., HANSON, M.A. 1995. The effects of anemia on heart, placenta and body weight, and blood pressure in fetal and neonatal rats. In *Journal of Physiology*, vol. 488,1995, p. 515-519.
7. DANKS, D.M. 1988. Copper deficiency in human. In *Anual Reviews of Nutrition*, vol. 3, 1988, p. 235-257.
8. FIELDS, M., LEWIS, C.G., BEAL, T., SCHOLFIELD, D. 1990. Copper deficiency in pregnancy: effect on maternal and feta polyol metabolites. In *Metabolism*, vol. 39, 1990, p. 531-537.
9. GIBSON, R.S. 1989. Assessment of trace element status in human. In *Progress in Nutrition Science*, vol. 13, 1989, p. 67-111.
10. HAWORTH, J.C. IINNI, I. 1980. Role of matallothionein in human placenta and energy consumption to infant size. In *American Journal of Obstetrics and Gynecology*, vol. 138, 1980, p. 1185-1189.
11. IYENGAR, G.V., RAPP, A. 2001. Human placenta as a ‘dual’ biomarker for monitoring fetal and maternal environment with special reference to potentially toxic

trace elements. Part 1: Physiology, function and sampling of placenta for elemental characterization. Vienna, Austria, (280) p.195-206.

12. KABATA–PENDIAS, A., PENDIAS, H., 1993. Biogeochemia pierwiastków śladowych. PWN. Warszawa.
13. KANTOLA, M., PURKUNEN, R., KROGER, P., TOOMING, A., JURAVSKAJA, J., PASANEN, M., SAARIKOSKI, S., VARTIAINEN, T. 2000. Accumulation of cadmium, zinc, and copper in maternal blood and developmental placental tissue: differences between Finland, Estonia, and St. Petersburg. In *Environmental Research*, vol. 83, 2000, p. 54-66.
14. KEEN, C.L., URIU-HARE, J.Y., HAWK, S.N., JANKOWSKI, M.A., DASTON, G.P., KWIK-URIBE, C.L., IINNI, I. 1998. Effect of copper deficiency on prenatal development and pregnancy outcome. In *American Journal of Clinical Nutrition*, vol. 67, p. 1003S-1011S.
15. KORPELA, H., LOUENIVA, R., YRJÄNHEIKKI, E., KAUPPILA, A. 1986. Lead and cadmium concentrations in maternal and umbilical cord blood, amniotic fluid, placenta and amniotic membranes. In *American Journal of Obstetrics and Gynecology*, vol. 155, 1986, p. 1086-1089.
16. KUHNERT, B.R., KUHNERT, P.M., LAZEBNIK, N., ERHARD, P. 1993. The relationship between placental cadmium, zinc, and copper. In *Journal of the American College of Nutrition*, vol. 12, 1993, p. 31-35.
17. KRACHLER, M., ROSSIPAL, E., MICETIC-TURK, D. 1999. Trace element transfer from the mother to the newborn-investigations on triplets of colostrums, maternal and umbilical cord sera. In *European Journal of Clinical Nutrition*, vol. 53, 1999, p. 486-494.
18. KWIK-URIBE, C.L., GIETZEN, D., GERMAN, J.B., GOLUB, M.S., KEEN, C.L. 2000. Chronic marginal iron intakes during early development in mice result in persistent changes in dopamine metabolism and myelin composition. In *Journal of Nutrition*, vol. 130, 2000, p. 2821-2830.
19. MOORE, K.L. 2003. The developing human. Clinically oriented embryology, 7th ed. Philadelphia: Saunders; Persaud TVN. 1-560.
20. PASTERNAK, K., FLORIAŃCZYK, B. 1995. Metale życia. Wybrane metale i ich rola w funkcjonowaniu organizmu człowieka. Wydawnictwo FOLIUM Lublin.
21. RAO, J., JAGADEESAN, V. 1996. Lipid peroxidation and activities of antioxidant enzymes in iron deficiency and effect of carcirogen feeding. In *Free Radicals in Biology and Medicine*, vol. 21, 1996, p. 103-108.

22. RAO, R., DE UNGRIA, M., SULLIVAN, D., WU, P., WOBKEN, J.D., NELSON, C.A., GEORGIEFF, M.K., 1999. Perinatal brain iron deficiency increases the vulnerability of rat hippocampus to hypoxic ischemic insult. In *Journal of Nutrition*, vol. 129, 1999, p. 199-206.
23. RICHTER, J., HAJEK, Z., PFEIFER, I., SUBRT, P. 1999. Relation between concentration of lead, zinc and lysozyme in placentas of women with intrauterine fetal growth retardation. In *Central European Journal of Public Health*, vol. 7, 1999, p. 133-139.
24. RIEMSCHNEIDER, R., MARTINS, A.F. 1978. Determination of microelements in placenta I Commun: spectrophotometric determination of copper content of human placentas. In *Geburtsh Universitat Frauenklinik*, vol. 38, 1978, p. 371-375.
25. ROSSIPAL, E. 2000. Investigation on the transport of trace elements across barriers in humans: studies of placental and mammary transfer. In *Journal of Trace Microprobe and Techniques*, vol. 18, 2000, p. 493-497.
26. SADLER, T. W. 1993. *Embriologia lekarska*. Warszawa: *Med Tour Press International*, 1993, p. 96-106.
27. SKY PECK, H.H. 1986. Trace metals and neoplasia. In *Clinical Physiology and Biochemistry*, vol. 4, 1986, p. 99-111.
28. SOEWINDO, S. 1995. The effect of iron deficiency and mental stimulation on Indonesian children's cognitive performance and development. In *Kobe Journal of Medical Sciences*, vol. 41, p. 1-17.
29. SORELL, T.L., GRAZIANO, J.H. 1990. Effect of oral cadmium exposure during pregnancy on maternal and fetal zinc metabolism in the rat. In *Toxicology and Applied Pharmacology*, vol. 102, 1990, p. 537-545.
30. SRIRAMACHARI, S., JAIN, A.K., DAS, S.K., BAPU, RAO S., SINDHU, J.K. 1997. Use of human placenta for environmental biomonitoring: Observations on multielemental analysis of placental tissue in a Delhi hospital. Harmonization of health-related environmental measurements using nuclear and isotopic techniques. Vienna: IAEA.
31. STEŃCZUK, W. 1990. *Toksykologia*. Warszawa: Wydanie Lekarskie PZWL, 1990.
32. SWANSON, C.A., KING, J. C. 1987. Zinc and pregnancy outcome. In *American Journal of Clinical Nutrition*, vol. 46, 1987, p. 763-771.
33. WALTERS, G.O., MILLER, F.M., WORWOOD, M. 1973. Serum ferritin concentration and iron stores in normal subjects. In *Journal of Clinical Pathology*, vol. 26, 1973, p.770-772.

34. WIER, P.J., MILLER, R.K., MAULIK, D., DI SANT'ACNESE, P.A. 1990. Toxicity of cadmium in the perfused human placenta. In *Toxicology and Applied Pharmacology*, vol. 105, 1990, p.156-171.

Contact address

Suprewicz, K., Uniwersytet Pedagogiczny w Krakowie, Instytut Biologii, 31-054 Kraków, ul. Podbrzezie 3, Poland, Email: k.suprewicz@interia.pl, iwona.kozikowska@gmail.com