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HIGH DOSES OF COPPER ADMINISTERED TO SHEEP AND THE POSTNATAL ADAPTATION OF THE TWIN LAMBS

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Abstract

The objective of this study was to determine the postnatal adaptation of 10 pairs of twin lambs after treating their mothers (n = 5) with 25 mg of copper as $CuSO_4 \cdot 5H_2O$ *per os* (twins I and II – experimental groups, each group n = 5) and 10 other ewes with saline (twins I and II – control groups, n = 5). Mothers were treated with copper daily for four weeks before conception, during pregnancy and for three weeks after parturition. Blood samples were taken from each lamb at birth, at 6, 12, 24 hours of age, and at 3, 7 and 21 days of age. Copper caused an increase (P<0.01) in ACTH concentrations after 24 hours of age in the group of experimental twins II compared to the group of twins I. However, a higher concentration of cortisol in the experimental twins I compared to twins II was noted. The total glucose concentration was lower in the group of experimental twins I compared to twins II (P<0.05) and in both (P<0.01) control groups. The results showed that during the first days of life, symptoms of copper poisoning occurred in the group of experimental twins I. In this case, paradoxically, the status of the second born twin was more advantageous in postnatal adaptation.

Key words: twin lambs, copper, cortisol, ACTH, toxicity

Copper is a cofactor for a number of enzymes and it is an essential trace element for all living organisms. In mammals, the uptake of copper occurs through the intestine in order to meet essential needs (Wijmenga and Klomp, 2004; Mendel et al., 2007). However, during pregnancy deficits of copper can result in the gross structural malformation of the conceptus. An excessive amount of copper in the fetus can also pose a risk. Acute toxicity of copper can result in a number of pathologies, and even death (Sargison et al., 1994; Hoffman, 2009). The danger of copper toxicity in livestock has been recognized and a greater sensitivity of sheep compared to other species has been acknowledged (Maiorka et al., 1998). It is already known that the placenta allows the transfer of copper from mother to the fetus. After birth, additional sources of copper to the lamb are in colostrum and milk (Silvestre et al., 2004; Bampidis et al., 2010).

Hormones released from developing hypothalamo-pituitary-adrenal axis (HPA) in fetuses help them prepare for an independent postnatal life. During pregnancy cortisol influences the development of digestive, blood vascular and endocrine systems (Sangild et al., 1995). Towards the end of a sheep's pregnancy, the increase in the amount of cortisol passed to the fetus initiates a succession of events which allow parturition to take place (Challis et al., 2000). In the fetus, cortisol affects the liver glycogen accumulation. This accumulation protects against hypoglycemia which is the greatest threat to the newborn (Edwards et al., 2001; Wang et al., 2006). The high level of cortisol in lamb blood plasma just after the birth gradually decreases during the first few hours of life (Wrońska-Fortuna, 2005). In the case of multiple pregnancies there is well-founded fear that the competition between the fetuses may inhibit their development and, as a consequence, reduce the efficiency of postnatal adaptation. Adverse intrauterine conditions may affect the fetus development and condition.

The present study attempted to compare the adaptation mechanisms of twin lambs. This was done by measuring the blood ACTH and cortisol concentrations as well as the levels of glucose and free fatty acids (FFA), after treating their mothers with toxic doses of copper.

Material and methods

Ten multiparous Polish Longwool ewes, 2 to 3 years of age with a mean body weight of 60±5 kg, were used in this study. Ewes were fed twice daily at 07:00 and 16:00 with a diet formulated to supply 100% of the nutritional requirements according to the National Research Institute of Animal Production recommendations, for maintenance (Norms, 1993). Water was available *ad libitum*. Ewes were lambed in February and ten pairs of spontaneously born twin lambs (twin I – first born, twin II – second born) of both sexes, were used for further experiment. Five pairs of newborn twin lambs from mothers which received *per os* 25 mg of copper as $CuSO_4 \cdot 5H_2O$ (50 ml), were studied (experimental group). The solution was given to the mothers every day from the first day of natural estrous cycle symptoms, and during the next three cycles, during the whole pregnancy and during the three weeks after parturition. The next five pairs of twin lambs were treated as the control group, and were born from untreated mothers (control group). Blood samples were taken from each lamb: the first time just after birth but before colostrum consumption, at 6, 12, 24 hours of life and at 3, 7 and 21 days of age.

In blood plasma, ACTH and cortisol concentrations were determined by RIA using commercial kits (Brahms, Hennigsdorf, Germany; DSL, Webster, USA, respectively). The sensitivity of the method was 2 pg/ml for ACTH and 5 nmol/l for cortisol. The level of glucose was determined by the enzymatic method using the Glukoza-Oxy kit (Pointe Scientific, Warsaw, Poland). Free fatty acid concentration was determined by the colorometric method according to Duncombe (1964).

Data were analysed using SigmaStat 2.03 (SPSS Science Software, Germany). Values were considered to be statistically significant at P<0.05 and highly significant at P<0.01.

Results

A similar concentration of ACTH in the plasma of the control group of twin lambs was found throughout the whole experiment except for the first 6 hours of life (Figure 1). The copper given to the experimental group mothers caused an increase (P<0.01) in ACTH concentrations after the first 24 hours of life in the group of twins II compared to twins I (Figure 2). The ACTH concentration in twins I from the experimental groups was also lower in comparison to the control twin group (P<0.01).

The highest concentration of cortisol in blood plasma was observed immediately after birth in all groups of newborn lambs (Figure 3). Cortisol concentrations were slightly higher but not significantly (P>0.5) and always visible in twins II of the control group. The long-time treatment of the mothers with toxic doses of copper caused a higher concentration of cortisol in the experimental group of twins I compared to twins II from the same group (Figure 4). Differences (P<0.05) between cortisol concentrations between twins I from the experimental and control groups were also noted (Figure 4).

The lack of differences in the glucose concentrations between the control groups of twins was noted (Figure 5). After birth, the concentration of glucose in the blood plasma of the experimental twin I lambs was higher (P<0.01) than in the blood plasma of experimental twin II lambs apart from data collected at 24 hours of life. The total glucose concentration was lower in the experimental group of twins I compared to twins II (P<0.05) from the same group, and compared to both (P<0.01) control groups of twins as well (Figure 6).

The highest level of FFA was observed during the first 12 hours of life in twins I from the control group (Figure 7). In twins II from the control group, however, FFA was higher (P<0.01) in comparison with the lambs from the experimental groups (Figure 8). The levels of FFA in both experimental twin lamb groups were different (P<0.05) (Figure 8). A large unexpected increase in FFA levels in twins II from the experimental group was visible after 6 hours of life. In the next hours and days the FFA level decreased and its values were similar in experimental lamb groups (Figure 7).

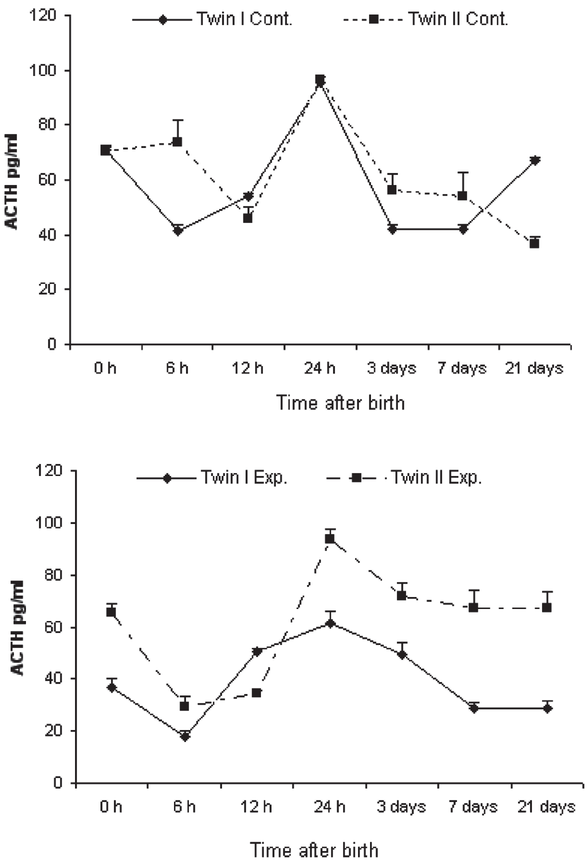

Figure 1. Mean (± SEM) ACTH concentrations in plasma of lambs collected on day of birth (0), at 6, 12, 24 h, and at 3, 7 and 21 days of life after treating mothers with saline (control groups twin I and II, panel A) or copper (experimental groups twin I and II, panel B)

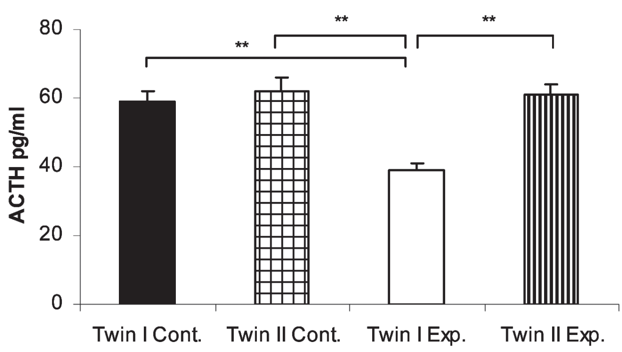

Figure 2. Mean (± SEM) ACTH concentrations in plasma of lambs from control and experimental groups of lambs, ** – P<0.01

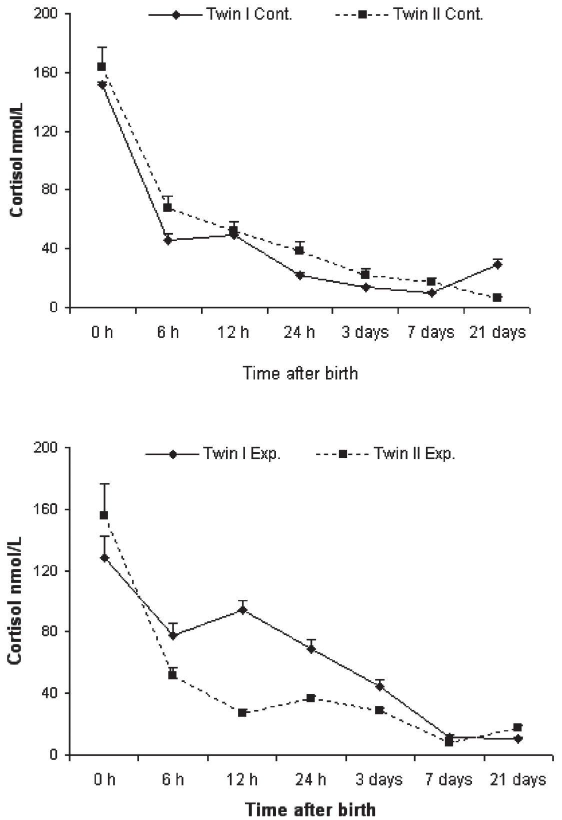

Figure 3. Mean (± SEM) cortisol concentrations in plasma of lambs collected at birth (0), at 6,12, 24 h, and at 3, 7 and 21 days of life after treating mothers with saline (control groups twin I and II, panel A) or copper (experimental groups twin I and II, panel B)

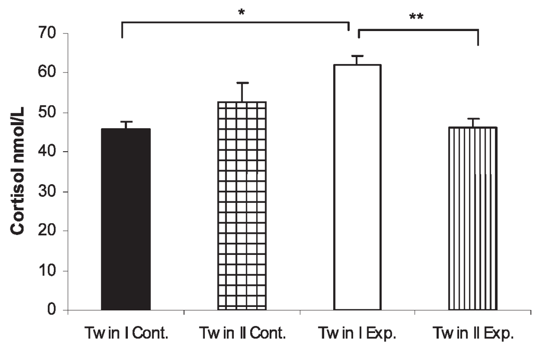

Figure 4. Mean (± SEM) cortisol concentrations in plasma of lambs from control and experimental groups of lambs,* – P<0.05, ** – P<0.01

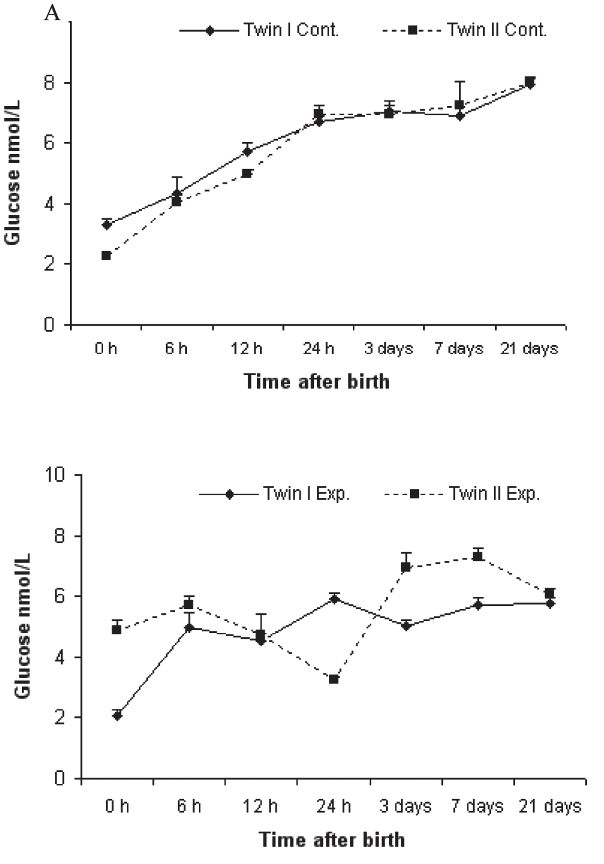

Figure 5. Mean (± SEM) glucose levels in plasma of lambs collected at birth (0), at 6,12, 24 h, and at 3, 7 and 21 days of life after treating mothers with saline (control groups twin I and II, panel A) or copper (experimental groups twin I and II, panel B).

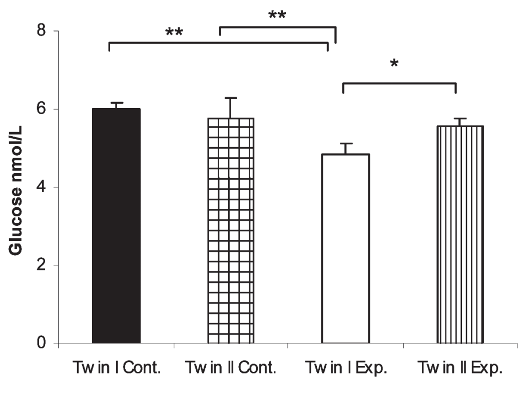

Figure 6. Mean (± SEM) glucose levels in plasma of lambs from control and experimental groups of lambs, * – P<0.05, ** – P<0.01, *** – P<0.001

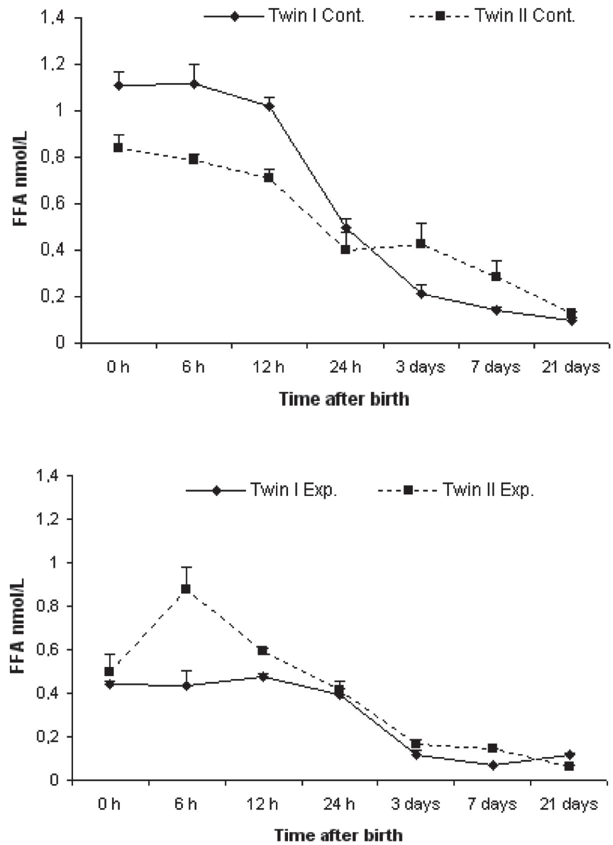

Figure 7. Mean (± SEM) FFA levels in plasma of lambs collected at birth (0), at 6,12, 24 h of life, and at 3, 7 and 21 days of life after treating her mother with saline (control groups twin I and II, panel A) or copper (experimental groups twin I and II, panel B)

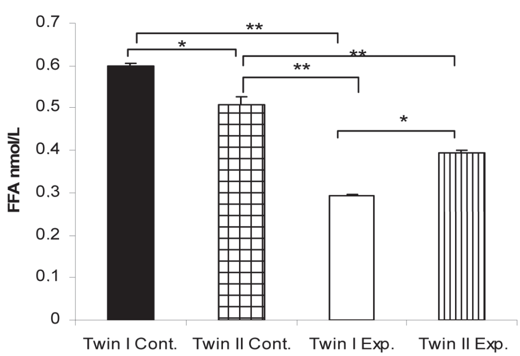

Figure 8. Mean (± SEM) FFA levels in plasma of lambs from control and experimental groups of lambs. * – P<0.05, ** – P<0.01, *** – P<0.001

Discussion

Results of the present study concerning the cortisol concentration in blood plasma provide evidence for a strong HPA activation in both twin I and II lambs just after birth. In near-term sheep, an increase in cortisol concentration in the blood of fetuses as a consequence of HPA activity augmentation has been observed. In a mother's organism the highest activity of HPA, which is manifested by the highest concentration of cortisol, was determined at the time of labour. Our study showed that during the first 6 hours of life a significant decrease in cortisol concentration in lambs was observed, particularly in twins I. Fetus growth and development takes place in the presence of low concentrations of cortisol, but during the late gestation the adrenal gland activates as a consequence of more and more uncomfortable intrauterine conditions. In twin pregnancies competition exists between the fetuses. Most likely the first twin may inhibit the development of the second one. As a consequence there is reduced efficiency of postnatal adaptation of that lamb. The predomination of the first twins also carries with it the risk of placenta transfer of dangerous and toxic substances, e.g. copper from the mother to the fetuses. The way in which copper is transferred during pregnancy is not clear. One hypothesis explains that in humans copper is transferred through the placenta in a concentration gradient-dependent mechanism (Nandakumaran et al., 2002). On the other hand, Al-Saleh et al. (2004) suggested a passive transport of this element. However, Krachler et al. (1999) showed twice as high a concentration of copper in human-mother blood plasma than in the umbilical cord. The authors' explanation is that the placenta inhibits the transfer of copper towards the human fetus (Krachler et al., 1999). In ovine pregnancy, fetuses are completely dependent on copper supplies from the mother and there is a significant correlation between copper concentration in sheep blood plasma of fetus and mother (Apostolakis et al., 1994). The results of many experiments have shown the presence of copper in colostrum and milk (Silvestre et al., 2001). In cows the highest concentration of copper was found in colostrum just after parturition (Kume and Tanabe, 1993). In the case of lactating sheep exposed to toxic doses of copper, some amount of this element penetrates into the milk posing an additional threat for newborn lambs (Matthews and Challis, 1995). Probably, copper administered in toxic doses to mothers makes its way into the milk and appears to be a factor which additionally activates HPA axis. The consequence of this fact was a higher cortisol release from the adrenal gland of twins I. Paradoxically, twins II which consumed less milk were less subjected to this factor. The direct effect on cortisol released from the adrenal gland is controlled by the pituitary ACTH. The results obtained confirmed the negative feedback between these two hormones in twins from the control group as well as in twins whose mothers were exposed to toxic doses of copper. A rapid increase in the cortisol concentration was observed just before parturition in sheep fetuses. Matthews and Challis (1995) showed that in sheep fetuses during the last part of pregnancy, the increase of plasma cortisol is accompanied by high plasma values of ACTH. Apostolakis et al. (1994) described the positive feedback between these two hormones which appears for a short time near delivery.

The success of postnatal adaptation in lambs is conditioned by amounts of glycogen and brown adipose tissue (BAT) stores accumulated during prenatal life. After the birth, this reserved supply of glucose and free fatty acids (FFA) which protect against hypoglycemia and hypothermia break up. The results obtained showed that immediately after the birth the concentration of glucose in twins I from the control group of lambs, was about 1 mmol/l higher in comparison to values noted in the control group of twins II. This points to the competition between twins during pregnancy. Competition results in smaller amounts of glycogen being accumulated by twins II. A consequence is a lower concentration of glucose in blood plasma. An additional factor which helps increase the glucose level during the first hours of life is regular colostrum drawing. In the experimental groups of lambs the blood plasma concentration of glucose in twins II just after the birth was paradoxically twice as high as the values noted in the twin I group. These significant differences indicate untimely glycogenolysis during prenatal life. It is well known that during pregnancy these processes do not take place in fetuses (Hay, 1995). The results of our experiment suggest a copper influence on the considerably faster enzyme activity of glycogenolysis in twins II. The high rate of this process during the first 24 hours of life of twins II, probably caused exhaustion of glycogen reserves, the visible consequence being the smallest concentration of plasma glucose in the first 24 hours. During the next days of postnatal life of twins I, the values of the glucose level were always lower in comparison with the other groups of lambs (control twins and experimental twins II) until day 21 of life. Sansinanea et al. (1996) claim that one of the symptoms of copper intoxication in sheep is a decrease in the blood plasma glucose level. The decrease can be as much as 35%. In that case it places twins II in a privileged position because the consumption of smaller amounts of colostrum and milk diminishes the exposure of copper toxic consequence.

Apart from glucose, an important source of readily assimilated energy are free fatty acids. Hydrolysis of BAT supplies them just after the birth and in this way protects against hypothermia which is another threat to newborn lambs. Higher values of FFA were observed in the control twin I group. The present experiment showed that twins in the experimental group had a rate of lipolysis distinctly lower than the rates from twins in the control group. The copper excess in the diet of the sheep during pregnancy and lactation probably disturbs the process of lipolysis and diminishes thermoregulatory possibilities in lambs just after birth. This would then bring about symptoms of hypothermia. Lower values of plasma FFA observed in twin I lamb group, whose mothers received toxic doses of copper, indicate a lack of BAT tissue. This may be the result of difficulty in transferring FFA towards the fetus. In women from highly polluted regions, i.e. copper mining regions, the placenta was found to be jeopardized by the toxic impact of copper. Zadrożna (2003) showed an abundance of mineral and fibrinoid deposits and lipid droplets. This produced a compensatory increase in the mother-fetus exchange area due to excessive proliferation of placental villi. Villi proliferation in turn decreased the intervillous space and thus the influx of indispensable maternal blood. The analysis of results obtained in both control groups of lambs I and II, showed that the main sources of energy, particularly during the first 24 hours of life, are FFA. For twin lambs exposed to toxic doses of copper, though, it could be glucose. Our suggestion is that BAT tissue formed during prenatal life may also accumulate copper. The distinctly lower rate of lipolysis in both twins I and II from the experimental groups indicates a copper block of mitochondrial oxidation and disturbance of thermogenic processes (de Jesus et al., 2001). The compensatory role of glucose, which is also presented in colostrum, maintains the rate of metabolism in neonatal lambs.

The results of this experiment showed that during the first days of the postnatal life of lambs, the symptoms of copper poisoning occur in the group of first twins. The symptoms of copper poisoning lead to a prolonged activation of HPA axis, and an unfavourable metabolic consequence as a result of the effects of cortisol. In this case, paradoxically, the status of the second born twin is more advantageous in postnatal offspring adaptation.

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Podawanie wysokich dawek miedzi owcom a poporodowa adaptacja jagniąt bliźniąt

STRESZCZENIE

Celem badań było określenie poporodowej adaptacji 10 par jagniąt bliźniąt po doustnym podaniu 25 mg miedzi w postaci $CuSO_4 \cdot 5H_2O$ ich matkom (n = 5) (bliźnięta I i II – grupy doświadczalne, w każdej grupie n = 5) oraz soli fizjologicznej 10 innym maciorkom (bliźnięta I i II – grupy kontrolne, n = 5). Matki otrzymywały dodatek miedzi codziennie przez okres czterech tygodni przed zapłodnieniem, podczas ciąży i przez trzy tygodnie po porodzie. Próbki krwi pobierano od każdego jagnięcia po urodzeniu, w 6., 12. i 24. godzinie życia oraz w 3., 7. i 21. dniu życia. Dodatek miedzi spowodował wzrost (P<0,01) stężenia ACTH po 24 godzinach życia u bliźniąt II z grupy doświadczalnej w porównaniu do bliźniąt I. Stwierdzono natomiast wyższe stężenie kortyzolu u bliźniąt I z grupy doświadczalnej w porównaniu do bliźniąt II. Całkowita koncentracja glukozy była niższa u bliźniąt I z grupy doświadczalnej w porównaniu do bliźniąt II (P<0,05) oraz w obydwu (P<0,01) grupach kontrolnych. Uzyskane wyniki wskazują na wystąpienie objawów zatrucia miedzią u bliźniąt I z grupy doświadczalnej w okresie pierwszych dni życia. Paradoksalnie, w tym przypadku adaptacja poporodowa przebiegała korzystniej u drugiego z narodzonych bliźniąt.