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**THE IMPORTANCE OF AMNIOTIC FLUID IN FETAL  
GROWTH AND DEVELOPMENT  
SUMMARY**

Scientific mentor

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## **SUMMARY**

Fetal growth and development is a complex, long-lasting phenomenon and can be influenced by several variables. Although electrolytes in amniotic fluid are present in small amounts, they are considered essential for the health and well-being of the fetus. Exact prenatal evaluation of the biochemical composition of amniotic fluid can assess overall health and fetal maturity. Correlations were made between amniotic fluid electrolyte concentrations and fetal development. Regular ions found in amniotic fluid include sodium, potassium, chloride, calcium, magnesium, phosphate, and bicarbonate. These amniotic fluid ions play an important role in a normal pregnancy and can help prevent and diagnose fetal or maternal pathologies early.

Human amniotic fluid is a dynamic environment that undergoes multiple changes for harmonious fetal development. The amniotic space first appears 7-8 days after fertilization, and then the amniotic fluid comes mainly from the maternal plasma that crosses the fetal membranes. Fetal urine first enters the amniotic space at 8-11 weeks of gestation, and in the second half of pregnancy, fetal urine becomes the main contributing factor to amniotic fluid. During this period, the fetal keratinization of the skin is complete, decreasing the transport of water on the skin and at the same time a decrease in the osmolality of amniotic fluid. During pregnancy, fluid volume is determined by various mechanisms, including fetal urine production, oral, nasal, tracheal, and pulmonary secretion, fetal swallowing, and intramembranous way contributions. Approximately in the 16th week, when the fetal kidneys begin to function, the fetal urine also contributes to the formation of fluid. At one time, amniotic fluid was thought to be composed entirely of fetal urine. The fluid is absorbed through the fetal tissue and skin. After 22-25 weeks of pregnancy, keratinization of the skin of the embryo occurs. When this process ends around the 25th week, the amniotic fluid is absorbed mainly by the fetal intestine for the rest of the pregnancy.

After another 10 weeks, the amniotic fluid contains protein, carbohydrates, lipids and phospholipids, urea and electrolytes, all of which help the fetus to grow. In the late stages of pregnancy, most of the amniotic fluid consists of fetal urine.

Abnormally large or small volumes of amniotic fluid have been shown to predict poor fetal outcomes; therefore, a normal amount of amniotic fluid volume is crucial for the healthy development of the fetus or embryo. Amniotic fluid has proven to be a major diagnostic

tool when monitoring the progression and health of a pregnancy. Clinicians can use what is called the amniotic fluid index or the deepest pocket.

At present, there is no doubt that the usefulness of reproductive processes depends directly on the intake of essential, significant micronutrients, some of which are in a protein-bound state, including enzymes.

Of particular importance are metal-containing proteins, as well as metal ions that intervene during pregnancy in various processes, on the one hand due to the need to ensure the needs of the mother's body, but also for the growth and development of the fetus. For the chemical reactions listed, some of the most significant trace elements are: zinc, copper, magnesium and also iron.

During pregnancy, the diet should meet the needs of both the fetus and the mother, whose harmonious development is closely linked to the provision of adequate amounts of essential elements, including iron, copper and zinc. Their pre-pregnancy levels can also be significant.

Providing the right amount of essential elements with a diet or supplement can reduce the risk of fetal malformation and premature birth, including multiple pregnancies - associated with a higher risk of perinatal complications. These complications are likely to cause premature birth, miscarriage, hypotrophy of one or both fetuses, preeclampsia, stillbirth, or fetal atrophy syndrome.

Abnormal amniotic fluid volumes have been shown to predict poor fetal outcomes; therefore, a normal amount of amniotic fluid volume is crucial for the healthy and harmonious development of the fetus or depends on the nutrition of the mother, including the embryo. The composition of amniotic fluid essential nutrients and also the mother's exposure to toxic substances. Hypotheses have been made that amniotic fluid proteins or peptides have a strong bioactivity on cell growth and proliferation and that they modulate the process of embryonic development. The role of oligoelements in amniotic fluid is not yet well defined. Because amniotic fluid is ingested by the fetus, it has been suggested that this fluid may be an important source of certain elements for fetal nutrition, being considered a valuable marker of prenatal mineral status and exposure to toxic metals.

Thus, amniotic fluid has proven to be a major diagnostic tool when monitoring the progression and health of a pregnancy. It is unanimously accepted by clinicians that they can use AFI or SDP. These measurements are part of the biophysical profile which consists of fetal tone, fetal respiration and a non-stress test. AFI and SDP are estimates of amniotic fluid volume based on ultrasound measurements. An AFI greater than 24 cm or an SDP greater than 8 cm is considered polyhydramnios, which is an increased amount of amniotic fluid. Polyhydramnios can cause complications such as obstruction of the gastrointestinal tract, genetic disorders, musculoskeletal disorders or congenital diaphragmatic hernias. The risk factors of polyhydramnios can be divided into 4 main categories: maternal, placental, fetal or idiopathic factors. In contrast, oligohydramnios is an AFI less than 5 cm or SDP less than 2 cm. Oligohydramnios can cause complications such as renal agenesis, genitourinary tract obstruction and IUGR. Clinicians can use hormones, peptides and proteins from amniotic fluid to detect the existence of genetic disorders.

A number of techniques and procedures have been introduced in prenatal diagnosis over the past 10 years. They are divided into invasive and non-invasive. Standard non-invasive methods are: serum markers, serum tests, ultrasound.

Serum markers are used as screening tests in the first and second trimesters of pregnancy: alpha fetoprotein (AFP); human chorionic gonadotropin (hCG); serum estradiol E3; pregnancy-associated plasma protein (PAPP-A).

Further studies on amniotic fluid are under ongoing research; it remains a vital substance needed for the survival of the embryo or fetus and a marker of fetal condition that helps clinicians make decisions about the presence and outcome of pregnancies.

Some studies have determined the levels of essential elements in amniotic fluid and tests have been performed to establish normal values for the first weeks of pregnancy. In other studies, it has been shown that heavy metal ions can accumulate in amniotic fluid from a very early stage of gestation; however, little is known about the influence of metals in amniotic fluid on fetal development or the long-term effects of this early exposure.

Also, the standards and reference ranges for the concentrations of elements in amniotic fluid according to gestational age are not clearly contrasted. Not all factors and their mechanism of action in amniotic fluid are known exactly. Little is known about the

relationship between the concentration of the elements in amniotic fluid and fetal development, thus remaining a major chapter of interest for future studies.

This thesis deals with a topic of great interest, both locally, regionally, nationally and internationally, namely the importance of amniotic fluid in the growth and development of the fetus. Research studies on this topic are numerous and also very different. The material is structured according to the rules of writing in three main parts: (1) the general part, (2) the special part and (3) the conclusions and own contributions. In the general part, the most recent aspects related to: (a) amniotic fluid and its role in fetal development - embryology and physiology of amniotic fluid, pathophysiology, clinical significance of amniotic fluid (b) chemical elements of amniotic fluid - Sodium, chlorine, potassium, calcium, magnesium, bicarbonate and phosphate ions, zinc, cadmium, copper, selenium, iron, nickel, arsenic, lead, metalestrogens (c) paraclinical investigations on amniotic fluid - clinical significance, ultrasound, amniocentesis are presented. The special part is structured in two major chapters, one chapter dedicated to each original research direction addressed: (a) Amniotic fluid analysis - material and methods, results, (b) discussions divided in turn into subchapters of great interest.

The theme approached is in line with international and national concerns because it addresses a topic that affects millions of women around the world, from different social backgrounds and needs to be addressed and adapted.

The specific objectives of the thesis were represented by: (1) rigorous documentation through various scientific databases - Pubmed, Science Direct, De Gruyter, Wiley Online Library and Cochrane Library in order to identify specific current information; (2) detection of heavy metals in amniotic fluid by flame atomic absorption spectroscopy (FAES).

This retrospective study was conducted at the Bega Maternity Hospital in Timisoara, Romania, between April 1st 2020 and April 1st 2021. The design of the study is in accordance with the Helsinki Declaration, which was approved by the Bega Maternity Ethics Committee under decision number 260 / 16JUL2021.

Two groups of pregnant women were analyzed. One batch contained 100 pregnant women hospitalized in the “Bega” Maternity Hospital in Timișoara, and the second batch



contained 60 pregnant women hospitalized in the Maternity Hospital of Petrosani Emergency Hospital. Informed consent was provided to all 160 individuals for the procedure and use of the data for research purposes. Amniocentesis was performed in all patients between 15 and 21 weeks. Patients between 37 to 41 weeks of gestation had amniotic fluid collected during birth (both naturally and by cesarean section) before rupture of the amniotic membranes.

Three groups of patients were formed, two with patients from Timișoara and one with patients from Petroșani. The group of pregnant women from Petroșani contains 60 patients whose gestational age is between 37 and 41 weeks. The groups in Timișoara each contain 50 patients, one containing patients whose gestational age is between 37 and 41 weeks, and the second containing patients whose gestational age is between 15 and 21 weeks.

The cases chosen for the study were those of mothers with a single fetal pregnancy. In addition, pregnancies of less than 15 weeks, pregnant patients with severe anemia, haematological, neoplastic, cardiac or metabolic disorders were excluded. Patients with previous perinatal complications or other proven fetal disorders were also excluded from the study.

The following clinical and demographic data were collected for each patient: patient age, gestational age, concentrations of Lead, Copper, Nickel, Cadmium, Arsenic, Zinc, Iron. (in mg / l), femur length (FL, in mm), area of residence (urban or rural) and smoking status (non-smoker, former smoker - either past smoker or patient who has given up this habit once pregnancy was suspected and / or confirmed by the gynecologist, and active smoker). The data was collected in a Microsoft Excel table.

Normal distribution was assessed using the Shapiro-Wilk test. Descriptive statistics for numerical variables include means (plus 95% confidence interval, CI95), standard deviations (SD), medians, and inter-quartile interval (IQR).

The t-test with two factor comparisons was used to compare variables that were normally distributed. This test was primarily used to compare demographics.

The Mann-Whitney U test (also known as the Wilcoxon rank-sum test) was used to compare the variables with the non-parametric distribution. This test was mainly used to compare the values of heavy metal ion concentrations in amniotic fluid.

For comparing the variables, frequency as a percentage (%) and / or number (n) was included. The alpha level was set at 0.05, and all p values <0.05 were accepted as statistically significant. All data was processed using the Statistical Package for Social Sciences (SPSS), version 22 for Windows (IBM, Armonk, NY, USA).

The average age of the patients from Timișoara, with a gestational age between 15 and 21 weeks was 27.76 (CI95: 26.67-28.85). The average for the weeks of gestation was 18.14 (CI95: 17.63-18.65). The length of the femur was on average 24.94 (CI95: 23.15-26.74).

The average age of the patients from Timișoara, with a gestational age between 37 and 41 weeks was 27.46 (CI95: 26.64-28.48). The average for the weeks of gestation was 38.66 (CI95: 38.36-38.96). The length of the femur was on average 74.55 (CI95: 73.79-75.30).

The average age of patients in Petroșani, with a gestational age between 37 and 41 weeks was 26.83 (CI95: 25.73-27.93). The average for the weeks of gestation was 39.07 (CI95: 38.70-39.43). The length of the femur was on average 76.13 (CI95: 75.50-76.75).

Analysis of pregnant women in Timisoara, Group I vs. Group II compares the concentration of heavy metal ions in amniotic fluid of pregnant women (15-21 weeks), who underwent amniocentesis, with those of women with advanced pregnancy (37-41 weeks), who were sampled during birth, before rupture of membranes.

Analysis of pregnant women from Timișoara and Petroșani, Group II vs. Group III compares the concentration of heavy metal ions in the amniotic fluid of women with advanced pregnancy (37-41 weeks), whose amniotic fluid was collected during childbirth, before rupture of the membranes. Thus, the city of Timișoara, which has a moderate degree of industry, is compared with the city of Petroșani, a town known for its mining and metallurgical industry.

From the demographic point of view, the gestational age of the patients from the Timisoara group was 38.66 (CI95: 38.36-38.96) on average, and for the patients from the Petroșani group was 39.07 (CI95: 38.70-39.43). When performing the t-test, the value  $p = 0.08415$  was returned, statistically insignificant.

The length of the femur was on average 74.55 (CI95: 73.79-75.30) within the group from Timișoara and 76.13 (CI95: 75.50-76.75) within the group from Petroșani. When performing the t-test, the value  $p = 0.00160$  was returned, statistically significant.

The average age of patients with a gestational age between 37 and 41 weeks in Timișoara was 27.46 (CI95: 26.64-28.48). and the average age of the patients from

Petroșani was 26.83 (CI95: 25.73-27.93). When performing the t-test, the value  $p = 0.40396$  was returned, statistically insignificant.

In the statistical analysis of heavy metal ion concentrations in amniotic fluid, the only heavy metals without statistically significant difference were nickel ( $p = 0.58320$ ) and iron ( $p = 0.90440$ ). In the group of pregnant women from Timișoara, a single ion was demonstrated whose concentration is higher than in the group of pregnant women from Petroșani, namely the body ( $p = <0.00001$ ). The concentration of lead ions ( $p = 0.03409$ ), cadmium ( $p = 0.00206$ ), arsenic ( $p = 0.00896$ ) and zinc ( $p = <0.00001$ ) were higher in the Petroșani group.

Intrucât fumatul poate afecta concentrațiile elementelor, cum ar fi ionii de metale grele, din probele de sânge, urină, păr și / sau pat unghial al piciorului, s-a evaluat și starea de fumător a pacienților.

Also, when we talk about endometriosis, an important aspect is represented by amniotic fluid. It also contains scaled endometrial cells that could migrate and implant in the adjacent tissues followed by the incision. As we have seen in previous chapters, it is known that heavy metal ions have strong oxidative abilities. In addition to this direct effect, they can also interact with estrogen, creating metalloestrogens, which could have a hormonal influence on rebellious endometrial cells.

The bioactive components found in amniotic fluid are important and can be monitored by amniocentesis. This tool allows healthcare professionals to assess the condition of a developing fetus. Common ions such as sodium, potassium or calcium have been extensively studied in the past.

Heavy metal ions require increased attention, as minimal differences in concentration could influence fetal development. Cadmium, lead and arsenic are elements with high toxicity and at most limited physiological function. Thus, it is advisable to avoid these elements, especially by pregnant women and children.

Metalloids, such as iron, copper, zinc, or nickel, should be discussed with pregnant patients by their gynecologist to see if supplementation is needed. Further and long-term research needs to be done to outline the effects of these elements, as well as how they affect prenatal outcomes and the development of the baby after birth.

A pathology on which the etiology of these elements can have a direct and indirect effect is endometriosis, especially the iatrogenic type. This is an entity that is underdiagnosed due to the non-specificity of its symptoms and signs, but also because it occurs at a different time from the original intervention. To avoid this, a high level of suspicion should be maintained in any woman who has abdominal or pelvic pain, most often after surgery.

There are no clear guidelines for diagnosing and managing cases of iatrogenic endometriosis. Their treatment remains in complete excision of the lesion and histopathological examination to confirm the diagnosis and rule out malignancy.