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Stereological Analysis of Adolescent Placentas and Anthropometric Characteristics of Newborns

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ABSTRACT

Introduction: Knowledge of the size of surfaces available for transport is important for assessing the amount of nutrients that can be transmitted to the fetus for its normal growth and development. **Aim:** The aim of our study, was to determine the stereological structural parameters of the parenchymal part of placenta, ratio of birth weight and placental weight, and to determine their correlation with the body length and head circumference of the newborns of adolescent pregnant women. **Methods:** The study was conducted on a total of 60 human placentas of term pregnancy, divided into two groups according to the age of pregnant women. The experimental group consisted of 30 placenta of pregnant women aged 13-19. The control group consisted of 30 placenta of pregnant women aged 20-35. Computer assisted morphological analysis of images of histological preparations using stereological methods was performed. **Results:** Surface density of terminal villi of adolescent placentas is significant higher than the control group ($t = 14,179$, $df = 29$, $p < 0,0001$). The T-test ($t = -5,868$, $df = 29$, $p < 0,0001$) showed statistically significant difference in the surface density of fibrinoid in two compared groups. T-test ($t = 6.438$, $df = 29$, $p < 0.0001$) found that total surface of terminal villi was significantly higher in adolescent placentas. The T-test ($t = -6,747$, $df = 29$, $p < 0,0001$) found that total surface of fibrinoid was significantly lower in adolescent group. The T-test ($t = 4.203$, $df = 29$, $p < 0.0001$) found that the ratio of birth weight of newborn and adolescent placental weight was significantly higher in relation to the control group. **Conclusion:** Adolescent placentas was more efficient in increasing the weight of newborns, compared to the control group placentas.

Keywords: adolescent pregnancy, placenta, stereology, neonates.

1. INTRODUCTION

Adolescent pregnancy has far-reaching consequences for the mother and child. Most research and studies from developed and developing countries provide consistent data that adolescent pregnancy brings increased risk for adverse pregnancy outcomes, especially for premature labor, births of low birth weight infants regardless of gestation age, intrauterine growth restriction, mortality at first year life, and various developmental disorders occur. The number of adolescent pregnancies in Tuzla Canton (and B&H) is increasing (1-3).

Additional aggravating circumstances are the result of well-known differences, especially between developed, underdeveloped and developing countries, such as poverty, lack of education, inadequate antenatal protection and limited access to contraception (1).

However, very few studies were devoted to histologic and stereological placental analysis in adolescent pregnancy and its impact on human pregnancy (4-6). The results of these few studies suggest an increase in the placenta efficiency that in adolescent pregnancies is trying to promote fetal growth and development by increasing its functional efficiency, but at the same time there are pronounced changes in placental structure.

The morphometric analysis of parenchymal part of the placenta, as a carrier of synthetic activity of organs in adolescent pregnancy, on the course and outcome of pregnancy have not been studied so far in our country. As the risk for children increases in adolescent pregnancies, knowledge of the size of surfaces available for transport is important for assessing the amount of nutrients that can be transmitted to the fetus for its normal growth and develop-

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ment, which is why we have decided for this research (4-6).

2. AIM

The aim of our study, was to determine the stereological structural parameters of the parenchymal part of placenta (surface density and total surface of terminal villi and fibrinoid), ratio of birth weight and placental weight (BW/PW ratio), and to determine their correlation with the body length and head circumference of the newborns of adolescent pregnancies.

3. METHODS

Material sampling and histological processing

The study of the morphological structure of placenta was performed at the Department of Histology and Embryology at the Faculty of Medicine, University of Tuzla. The study was conducted on a total of 60 human placentas of term pregnancy, divided into two groups according to the age of pregnant women. The experimental group consisted of 30 placenta of pregnant women aged 13-19. The control group consisted of 30 placenta of pregnant women aged 20-35.

From each examined placenta, the amniotic membranes and umbilical cord were first removed. The placental weight was determined by weighing on the digital weigh, the measured weight being rounded to the nearest 1 gram value.

For histological processing, tissue samples were taken through the thickness of the entire organ, from the chorionic to the basal plate (parenchymal part). From each placenta five tissue samples were taken dimension of 1 cm³. The tissue was fixed in a 10% aqueous neutral formalin solution, molded into paraffin and cut into cuts of 8 µm thickness. The deparaffinized cuts are colored by hemalaun and eosin (HE).

Stereological analysis

For this research, the compiled hierarchical model of the placenta contained a detailed parenchymal part of the organ that was the subject of quantitative analysis.

Photographing histological preparations was done. For this purpose, the research microscope with an integrated digital camera was used. On the same microscope, calibration was performed using digital imaging software. Analysis of histological compositions was done on magnification 10x.

Computer assisted morphological analysis of images of histological preparations using stereological methods was performed. For the stereological analysis, the reference area was the parenchymal part of the placenta: terminal villi and fibrinoid.

The size of the sample, or the required number of stereological measurements for each of the investigated variables in both groups of pregnant women, is determined by the De Hoff procedure.

Stereological analysis was carried out by the multipurpose test system M42, using a 10x lens. The quantitative analysis included the relative and total variables of the relevant structures.

Newborns

A total of 300 newborns were divided into two groups. The first group consisted of 150 newborns of mothers aged 13-19. The second group consisted of 150 newborns of mothers aged 20-35. The body weight of newborns was measured immediately after birth with the mechanical weight, where the weight was rounded to the nearest 10 grams.

The body length was measured by centimeter band immediately after birth. The measured distance of the vertex-heel with fully extended feet is measured and the measured value is rounded to the nearest full number. The head circumference was measured by a centimeter band at the fronto coccal plane immediately after birth.

All planned measurements were made according to the provisions of the International Biological Program, anthropological methods and standardized instruments.

The survey was approved by the Ethics Committee of the UCC Tuzla.

Statistical analysis

During the statistical data processing, a t-test (mid-range comparison test), χ^2 -test (frequency comparison test), and z-test (proportionality test) were used. The value of $p < 0.05$ was considered to be a statistical significance indicator.

4. RESULTS

Relative stereological variables

Surface density of terminal villi

Surface density of terminal villi of adolescent placentas (Svtva) ranged from 25,396 to 40,507 mm⁻¹ (average of 31,212 ± 3,816 mm⁻¹). Surface density of terminal villi of control group placentas (Svtvc) ranged from 13,841 to 24,126 mm⁻¹ (average of 19,276 ± 2,33 mm⁻¹).

The values of the surface density of terminal villi (Svtv) for each explored placenta are shown in Figure 1. Statistical analysis of t-test results shows that the Svtva is significant higher than the control group ($t = 14,179$, $df = 29$, $p < 0,0001$).

Surface density of fibrinoid

Surface density of fibrinoid of the adolescent placentas (Svfa) ranged from 0 to 2,031 mm⁻¹ (average of 0.926 ± 0.569 mm⁻¹). Surface density of fibrinoid (Svfc) of the control group placentas ranged from 0.888 to 2.031 mm⁻¹ (average of 1.443 ± 0.313 mm⁻¹). T-test ($t = -5,868$, $df = 29$, $p < 0,0001$) showed statistically significant difference in the Svfa compared to Svfc.

Absolute stereological variables

Total surface of terminal villi

Total surface of terminal villi of adolescent placentas (Stva) ranged from 9.49 to 21.375 m² (average of 14,937 ± 2,823 m²). Total surface of the terminal villi of control group placentas (Stvc) ranged from 8.373 to 14.411 m² (average of 10,856 ± 1,662 m²).

Total surface of terminal villi (Stv) are shown in Figure 2. T-test ($t = 6.438$, $df = 29$, $p < 0.0001$) found that the Stva was significantly higher than the Stvc.

Total surface of fibrinoid

Total surface of fibrinoid adolescent placentas (Sfa) ranged from 0 to 1,158 m² (average of 0.456 ± 0.31 m²).

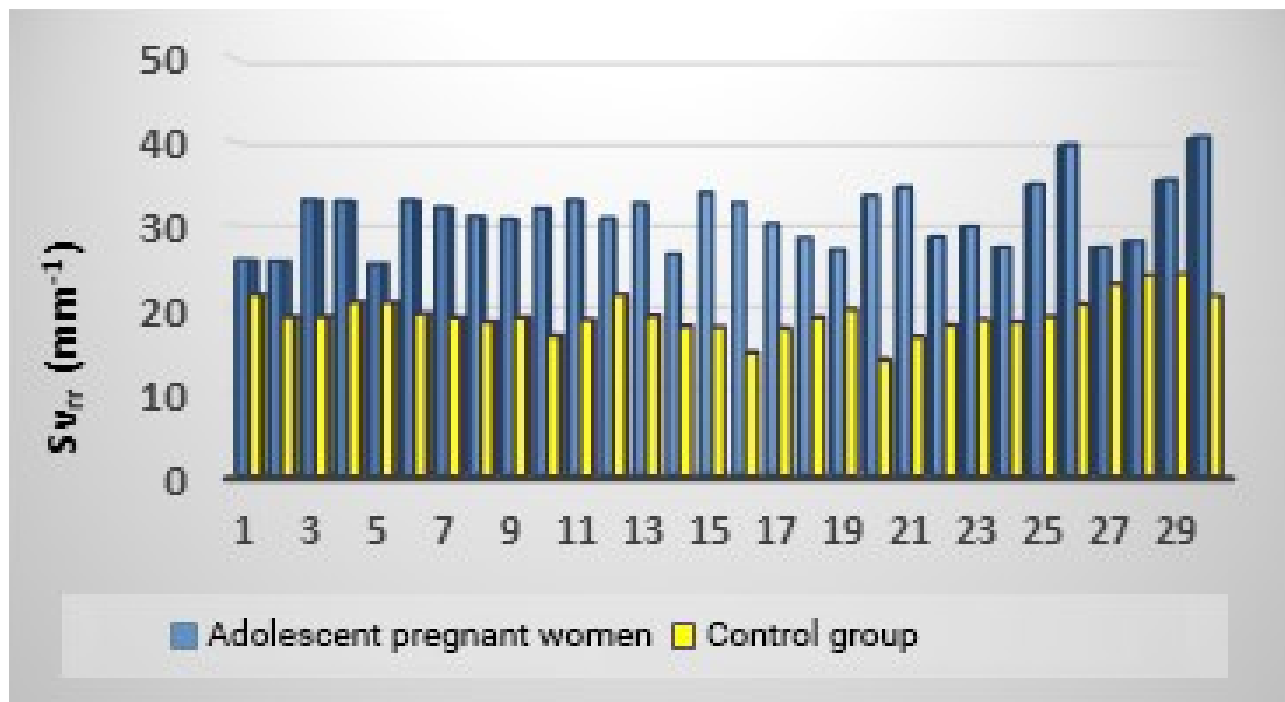


Figure 1. The mean value and standard deviation of the surface density of terminal villi of examined placentas

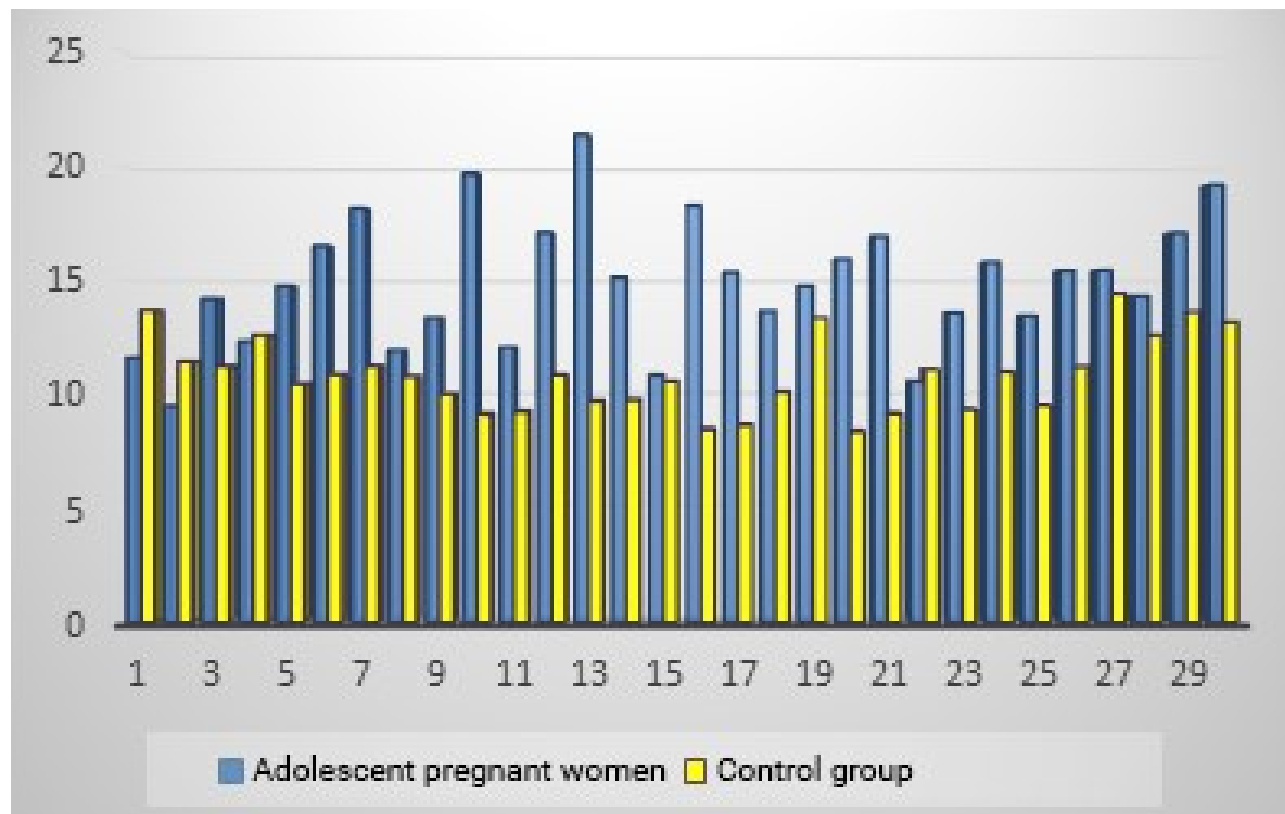


Figure 2. Values of total resorption of terminal villi placenta of adolescent pregnant women and control group

Total surface of fibrinoid of control group placentas (Sfc) ranged from 0.444 to 1.2 m² (average of 0.812 ± 0.188 m²). T-test (t = -6,747, df = 29, p <0,0001) found that the Sfa was significantly lower in compared to the Sfc.

Ratio of birth weight of newborns and placental weight

Ratio of birth weight of newborn and placental weight (BW/PW) of adolescent pregnancies ranged from 4,489 to 9,305 g (average of 7.06 ± 0.946 g), and in the control group ranged from 4,180 to 7,647 g (average of 5.993 ± 0.78 g). T-test (t = 4.203, df = 29, p <0.0001) found that

Characteristics	Newborns of adolescent pregnancies	Newborns of control group
Average body length in cm X± SD	52 ,25 ± 2,78	53 ,97 ± 2,37
Average head circumference in cm X± SD	32 ,80 ± 2,57	34 ,45 ± 2,89
TOTAL	150 (100)	150 (100)

Table 1. Average body length and head circumference of examined newborns

the BW/PW ratio was significantly higher in adolescent pregnancies in relation to the control group.

Body length and head circumference of newborns

Results of the study of body length and head circumference of newborns of adolescent pregnancies and newborns of the control group are presented in Table 1. Body length of newborns of adolescent pregnancies is 1.72 cm lower than the newborns in control group. The difference is statistically significant ($t = -4,195$; $p < 0,00001$).

The average size of the head circumference of the newborns of adolescent pregnancies is smaller by 1.65 cm compared to the control group ($t = -5.156$, $p < 0.00001$).

5. DISCUSSION

For our research, we have been encouraged by the fact that there is a small number of studies on the quantitative analysis of adolescent pregnancies in the literature (1-7). This research was carried out in order to determine the influence of adolescent age on the placenta structure, the course of pregnancy and the condition of newborns at birth.

The outcome of pregnancy and the condition of newborns are negatively influenced by structural pathological changes in placenta (7). The largest number of studies in the past concerned studies on adolescent pregnancy outcomes, and studies involving the structural quantification of placental morphology were negligible (8-10).

The placenta is a highly efficient multifunctional organ that integrates the upcoming mother and fetal signals to respond to the growing requirements in fetal growth. The modified placenta function in adolescent pregnancy is also reflected on the morphometric properties of the placenta (7, 9, 10). It is important to point out that most authors who have studied adolescent pregnancy and its influence on placental function have only analyzed relative and total parameters in a small extent and mostly studied individual stereological variables. In their studies, they showed a negative effect of gynecologic age on the villous / capillary surface area and reduced surface density of terminal villi in adolescent pregnancies with and without intrauterine growth restriction (IUGR) (4, 9-11).

Some studies have shown that the young age of adolescent pregnant women did not affect the morphometric variables of placentas. There was no statistically significant influence on the surface density of the terminal villi in relation to the control group (12), which is contrary to our results. In one study, quantitative analysis of placenta

of newborns with IUGR showed an increase in surface density of fibrinoids (Svf), with reduced placental weight (12, 13). Svf in our studies in adolescent placentas was lower with the statistically proven significant difference of 0.517 mm⁻¹, compared to the control group.

Much better and more important indicators of overall growth and structural adjustment of placenta are total values of stereological variables, unlike relative variables that represent indirect indicators of these changes. The full understanding of pathological structural changes requires a complete and common observation of all relative and total stereological variables in one place (13, 14).

When we look at them outside of the terminal villi, surface and volume depend on each other, because the increase in volume also leads to an increase in surface area, although in normal pregnancy the increase in the surface of terminal villi is more pronounced in relation to the increase in volume, which is reasonable because the increase in surface parameters simultaneously increases the total surface of the vasculosinital membrane (13-15). Surface densities and total surface of terminal villi of adolescent placentas (Stva) were significantly higher in relation to the same variables in the control group. There was a marked increase in the Stv occupying most of the parenchymal part of the placenta in an attempt to provide an optimal maternal-fetal exchange. Such a sharp increase in the surface represents the compensatory response to placental hypoxia (7).

Fibrin's deposits result is a cascade of blood coagulation reactions, and its role is to fill the cracks created on the surface of the syncytiotrophoblast. It can be found in every term placenta and is not a pathological finding, except when it is in a larger quantity (6, 7). One study that included quantitative analysis of placenta of newborns with IUGR demonstrated an increase in the total surface of fibrinoid (Sf) of the parenchymal part of placenta (6), which is contrary to our results.

Significantly lower Sfa is explained by a significantly higher number of preterm birth in a group of adolescent pregnancy with an average duration of 35,428 gestational weeks, because of that there was no time for this degenerative process to develop until the end of pregnancy to a greater extent (7).

The already mentioned causes of utero-placental insufficiency occurring in adolescent pregnancy can lead to placenta ischemia. This condition threatens the structural and functional placental reserves, which leads to reduced oxygenation and reduced transport of the fetus. The placental weight is in direct and significant correlation with the weight of the newborn, an indicator of intrauterine growth (7, 9). It should also be noted that the relationship between the weight of the newborn and placental weight was also analyzed in the study, which gives us an insight into the functional efficiency of the placenta and shows the increase of the weight of newborns in grams versus 1 gram of placenta. This is an feto/placental quotient, and the optimal values for this normal pregnancy count are from 6.8 g to 7.5 g (15, 16).

Several morphometric studies have demonstrated significant and positive correlation between weight of the

placenta with weight of newborns and length of gestation (7, 15-18).

In the study Hayward et al, the authors showed that the young age of adolescent pregnant women did not affect the placental weight (12). Some of studies have shown a negative impact of adolescent pregnancy on placental weight, weight of newborns, as well as association with low birth weight, lower Apgar score and premature rupture of membranes (7, 9, 19-21).

Our results show that placental grams of adolescent pregnancies led to an increase in weight of newborns of 7,06 g and in control group the weight gain in newborns per gram of placenta was 5.99 g ($p < 0.0001$). It can be concluded that adolescent placentas was more efficient in the increased weight of newborns, compared to the control group placentas.

Based on the analysis of the above results, we concluded that the outcome of adolescent pregnancy for newborns was relatively favorable, but at the same time there was a maximum exhaustion of compensatory mechanisms of placenta, reflecting on the weight of these placenta as well as on the stereological variables already shown (7). Growth of the human fetus is determined by growth support that depends on the ability of the mother to offer nutrients and oxygen, and from placental efficacy to fetch these nutrients to the fetus (14). Postnatal assessment of intrauterine growth of live neonates is performed on the basis of standards that are specific to each environment. In our study for the evaluation of intrauterine growth of newborns, we used the percentile growth curves for the Tuzla Canton (22, 23).

Adolescent pregnancy can adversely affect the important anthropometric characteristics of the newborn, body length and head circumference. Extreme reduction of anthropometric measurements (below optimal values) occurs when intrauterine stress lasts long enough (23, 25). The outcome of adolescent pregnancy and postpartal condition of newborns was investigated in various studies, which demonstrated a reduction in body length and head circumference of the newborns of adolescent pregnancies (7, 25, 26). In contrast to other studies, in one hospital-based study, it was determined that teenage mothers were not associated with worse maternal outcomes (27, 28). Studies from more economically advantaged clinics in developed countries yielded no increased obstetrical problem among adolescent mothers (27-29). Based on the statistical significance of the difference in the size of the head circumference and the body length between the investigated groups in our study, it is evident that there is an adverse influence of adolescent pregnancy and intrauterine stress on the birth characteristics of the newborn, which began early and lasted long.

Our results show that the average values of birth weight and head circumference are still within the optimal values for these parameters (7, 14, 22, 23), indicating the successful action of compensatory mechanisms, primarily from the placenta. The obtained quantitative parameters can serve as a basis for further conclusions on the structure, function and compensatory placement

mechanisms, as well as on the existence of pathological structural changes.

6. CONCLUSION

Adolescent placentas have significantly lower surface density of fibrinoid and significantly higher surface density of terminal villi. Adolescent placentas have a significantly higher total surface of terminal villi and a significantly lower total surface of fibrinoid. Adolescent newborns have optimal body length and head circumference. The weight of newborns per gram of placenta is significantly higher in adolescent pregnancies. It can be concluded that adolescent placentas was more efficient in increasing the weight of newborns, compared to the control groups placentas.

Author's contribution: S.M. and Z.Z. gave substantial contribution to the conception and design of the work and in the acquisition, analysis and interpretation of data for the work. Each author had role in drafting the work and revising it critically for important intellectual content. Each author gave final approval of the version to be published and they agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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