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ORIGINAL ARTICLE

Influence of Advanced Maternal Age and Gestational Age on the Morphology of Human Placenta

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Abstract

Background/Aim: The placenta is an extraembryonic organ necessary for foetal development. Due to its availability and high content of stem cells and growth factors, placenta tissue has found its application in regenerative medicine. The aim of this paper was to determine whether the age of the pregnant woman or the gestational age affects the morphology of the term placenta and whether placentas of advanced maternal age are suitable for application in regenerative medicine.

Methods: In this research 30 placentas of healthy pregnant women, aged from 18 to 42 years and from 36 up to 41 weeks of gestational age were used for analyses. Tissue samples were stained with standard haematoxylin and eosin staining and immunohistochemical staining with anti-CD34 antibody. The parameters of volume density of chorionic villi, intervillous spaces and fibrinoids, as well as the thickness of the placental barrier were determined.

Results: The volume densities of chorionic villi and fibrinoids were higher in placentas of advanced maternal age, as well as in placentas of gestational age from 38 to 41 weeks of gestation, while the volume of intervillous spaces was lower in these groups. With increased maternal and gestation age, the placental membrane thickens.

Conclusion: When sampling placentas for regenerative medicine purposes, only the placentas from pregnant women younger than 35 years of age and whose gestational age are not exceeding 38 weeks should be selected.

Key words: Placenta; Maternal age, advanced; Morphometry.

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Introduction

The placenta is an extraembryonic temporary organ that develops in the uterus of a pregnant woman with the aim of establishing a unique exchange of substances between the mother and the foetus. It is the only organ that consists of two tissues of different origins, the tissue of the mother and the tissue of the foetus. Maternal tissue or decidua basalis consists of large eosinophilic decidual cells surrounded by fibrinoid. The foetal part consists of the chorionic plate, chorionic villi and the spaces between the villi that are filled with maternal blood.[1, 2] In addition to being necessary for foetal growth and development, placental tissue has found its place in experimental and regenerative medicine.[3]

In recent years, there has been a great interest in the use of placental tissue in regenerative medicine due to the fact that placental tissue contains a large number of stem cells and growth factors

and it is also easily available after childbirth. With this interest new protocols for placental tissue sampling are being developed and new criteria are being introduced for the selection of optimal placental tissue since not every placenta is considered adequate. In addition to the already well-known and standardised application of amnion in wound healing, other parts of the placenta are also used in various forms for regenerative purposes.[4-6] It is worth emphasising that only healthy placenta can be used for these purposes. Disorders in the structure and function of the placenta lead to disorders in the growth and development of the foetus. Impaired health of the pregnant woman affects the structure and function of the placenta, whether it is infectious diseases or chronic non-communicable diseases such as diabetes or hypertension that are widely present in the population. Such pregnancies are marked as risky and require intensive monitoring.[2, 7]

It is a well-known fact that as a woman's age increases, so does the possibility of infertility and if pregnancy occurs, it is considered as risky pregnancy. In pregnancies at advanced maternal age, there is an increased risk for placental dysfunction, preeclampsia, reduced foetal growth or premature birth.[8, 9] It is well known that pregnant women aged 35 and over at the time of delivery are designated as pregnancies at advanced maternal age.[10, 11] In recent years there is a noticeable trend of getting married later and as a result the age limit for having offspring is shifting and there are more and more pregnancies at advanced maternal age today.

Although pregnancy at advanced maternal age is associated with numerous complications, there are just a few studies that have dealt with the morphology of the human placentas of pregnancies at advanced maternal age.[12] Additionally, numerous studies have shown that chronic diseases such as diabetes affect the morphology of the placenta and that this cannot be used as a biological material for regenerative purposes.[2, 13]

The aim of this study was to analyse the morphology of the placenta in healthy pregnant women in order to determine whether the age of the pregnant woman and the gestational age affect the morphology of the term placenta, as well as to determine whether these can be used as criteria for selection of placental tissue as biological materials in regenerative medicine.

Methods

Selection of placentas

The study was performed in accordance with the standards set by the latest revision of the Declaration of Helsinki. After obtaining the consent of the competent Ethics Committee, (decision No: 18/4.167/21), placentas of gestational age from 36 to 41 weeks of gestation were sampled in the Clinic of Gynaecology and Obstetrics of the University Clinical Centre of the Republic of Srpska, Banja Luka (UCC RS). Only placentas from healthy pregnancies that ended by vaginal delivery were included in the study. In order to avoid the influence of certain diseases on the morphology of the placenta, only pregnant women who had no history of cardiovascular diseases, diabetes or any of the infectious conditions during pregnancy were included in the study. According to the maternal and/or gestational age the placentas were divided into two groups. The control group (CON) consisted of the placentas of postpartum women who were under 35 years old and the second group consisted of postpartum women over 35 years old (AMA) and considered as advanced maternal age group. According to gestational age, they were younger (CON) or older than 38 weeks of gestation (OGA). There were 15 placentas in each group from which tissue was further sampled.

Tissue sampling and histological staining

From all placentas at the middle distance from the umbilical cord and the edge of the placenta, a 2 cm section of tissue covering the full thickness of the placenta, from the chorionic to the basal plate, was sampled. After 48 h of fixation in 4 % formaldehyde, tissue samples were processed in a Leica tissue processor and embedded into paraffin blocks. All samples were cut at a thickness of 5 micrometres and stained by routine staining with haematoxylin and eosin, as well as by immunohistochemical staining with CD34 monoclonal antibody (Anti-CD34 monoclonal antibody (QBEND-10), dilution 1:100, *Abcam,* Cambridge, United Kingdom). Antibodies were unmasked by boiling for 20 min in a citrate buffer with a pH value of 6. Endogenous peroxidase activity was blocked by incubating the tissue for 10 min with 3 % hydrogen peroxide (*Abcam*). Non-specific background staining was blocked by the ultra-vision block. At room temperature for 30 minutes, it was incubated with the primary antibody. For

visualisation the HRP/DAB IHC detection system (*Abcam*) was used. Mayer's haematoxylin was used for contrast staining. The analysis of the obtained samples was performed using a binocular Leica DM 6000 microscope, equipped with a Leica DFC310FX camera.

Morphometry analyses

Using *ImageJ* (version 18.0) the volume density of chorionic villi (VVchr), intervillous space (VVivs) and fibrinoids (VVfr), as well as the thickness of the placental barrier were determined. Volume density is a measure of the percentage representation of the examined placental structures in relation to the entire placental tissue. The number of examined visual fields (N) was determined according to the formula $N = (20 \times SD/X)^2$, where SD is the standard deviation and X is the mean value of the results obtained in a pilot study on 20 visual fields.[14] Volume density (VV) was determined as the quotient of points falling on the examined morphological element (Vf) with the total number of points of the test system (Vt), ie VV = Vf / Vt.

Statistical analyses

The statistical software package *Rcmdr* (version 2. 8-0) was used for statistical analysis. All results were considered statistically significant if $p < 0.05$ and highly statistically significant if $p < 0.001$. In the examples where highly statistically significant results were obtained, the level of statistical significance (< 0.001) was recorded. Given that numerical data were analysed, the coefficient of variation (CV ≤ 30 % - homogeneous data, normal distribution), values of skewness (from -3 to +3 - normal distribution) and kurtosis (from -1 to +1) were used to determine the normality of the distribution.

Results

The results showed that third trimester placentas, ie mature placentas, mostly contained intermediate and terminal chorionic villi. This type of villi had a smaller stroma and a larger number and volume of blood vessels in order to be able to meet the increase in exchange of substances that is needed for a larger fruit. On the surface of the chorionic villus, there were trophoblast cells, which were separated from the mesenchyme by the basement membrane. In this period of development, most of the villi are covered with syncytiotrophoblasts, so a greater number of syncytial nodes could be seen. Syncytiotrophoblast represents a continuous layer of cytoplasm with a larger number of nuclei. A larger amount of fibrinoids was also present in mature placentas (Figure 1).

The VVchr of placenta of control group, less than 38 weeks of gestation, ranged from 27.9 % to 43.5 %, while in placentas of older gestational age it was higher and ranged from 43.3 % to 63.6 % (CON: mean ± SD = 37.9 ± 4.9, OGA: mean ± SD = 55.6 ± 4.6). The VVivs of placentas with control group ranged from 56 % to 69.6 %, while in the older gestational age group it was from 32.2 % to 52.9 % (CON: mean ± SD = 61.4 ± 4.9; OGA: mean ± SD = 39.2 ± 5.3). The VVfr of control group placentas ranged from 0.3 to 1.1 % and in the older gestational age group it ranged from 2.0 to 11.2 % (CON: mean ± SD = 0.7 ± 0.2; OGA: mean ± SD = 5.1 ± 2.5). There was a statistically significant difference between the examined groups (Figure 2).

The VVchr of placentas in pregnant women under the age of 35, control group (CON) ranged from 28.9 % to 63.6 % and in pregnant women with advanced maternal age (AMA) from 29.7 to 58.5 (CON: mean ± SD = 48.8 ± 10.2, AMA: mean ± SD = 42.4 ± 10.1). The VVivs in the control group ranged from 38.7 % to 64.4 % and in the advanced maternal age group from 32.5 % to 69.6 % (CON: mean ± SD = 47.9 ± 12; AMA: mean ± SD = 55.4 ± 12.5). The VVfr of pregnant women in the control group ranged from 0.9 % to 9.2 % and in AGA from 0.4 % to 11.6 % (CON: mean ± SD = 3.4 ± 2.5, AMA: mean ± SD = 2.2 ± 3.1). There was no significant statistical difference between these two groups (Figure 2).

The average value of the thickness of the placental barrier in placentas with a gestational age of less than 38 weeks, control group was 5.07 µm and placentas with an older gestational age was 5.94 µm, CON: mean ± SD = 5.1 ± 0.2, OGA: mean ± SD = 5.9 ± 0.5. There was a significant statistical difference between these two groups (Figure 3 and 4).

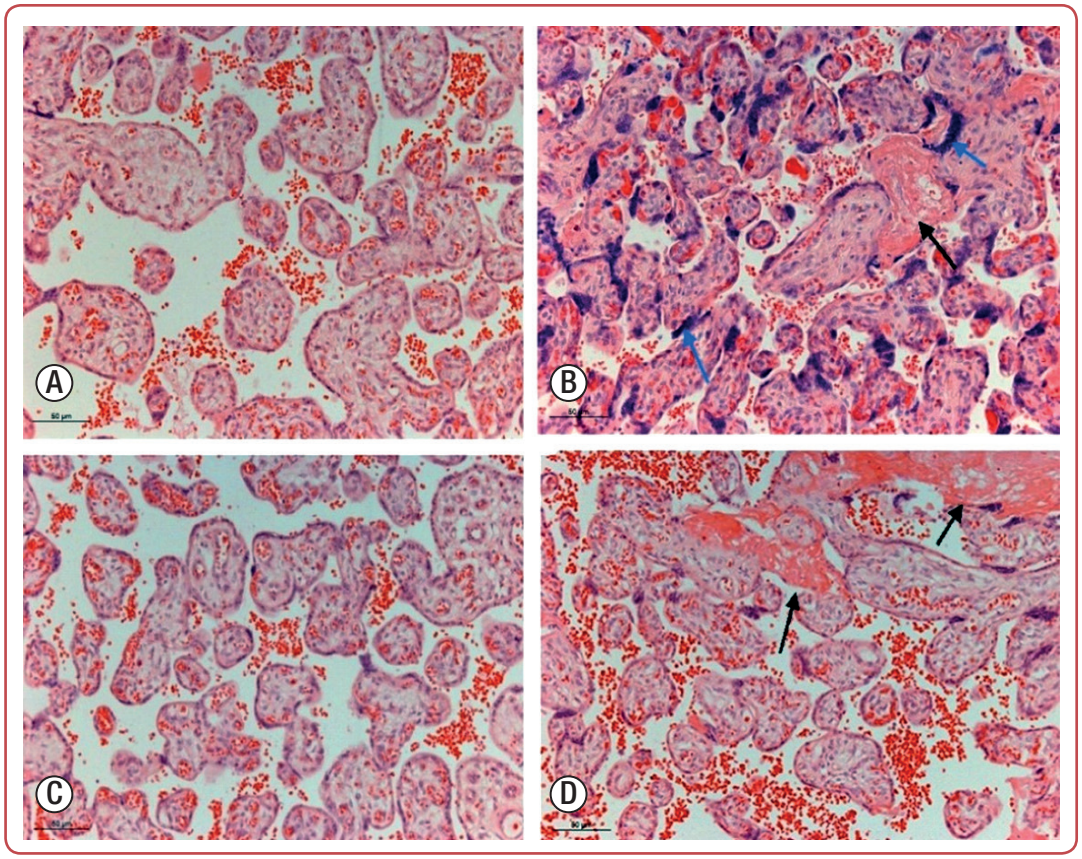

Figure 1: Chorionic villi of the placenta, haematoxylin and eosin (H & E) stain, magnification 20 x, scale bar 50 µm; A) control group, gestational age of below 38 weeks of pregnancy, B) older gestational age, gestational age of more than 38 weeks of pole pregnancy, C) control group, maternal age below 35 years, D) advance maternal age.

The image shows more compact structure of advanced maternal age placenta and older gestational age placenta (gestational age of more than 38 week), decrease in volume of intervillous space, increase in the volume of chorionic villi and fibrinoid (black arrow), presence of abundant syncytial nodes (blue arrow).

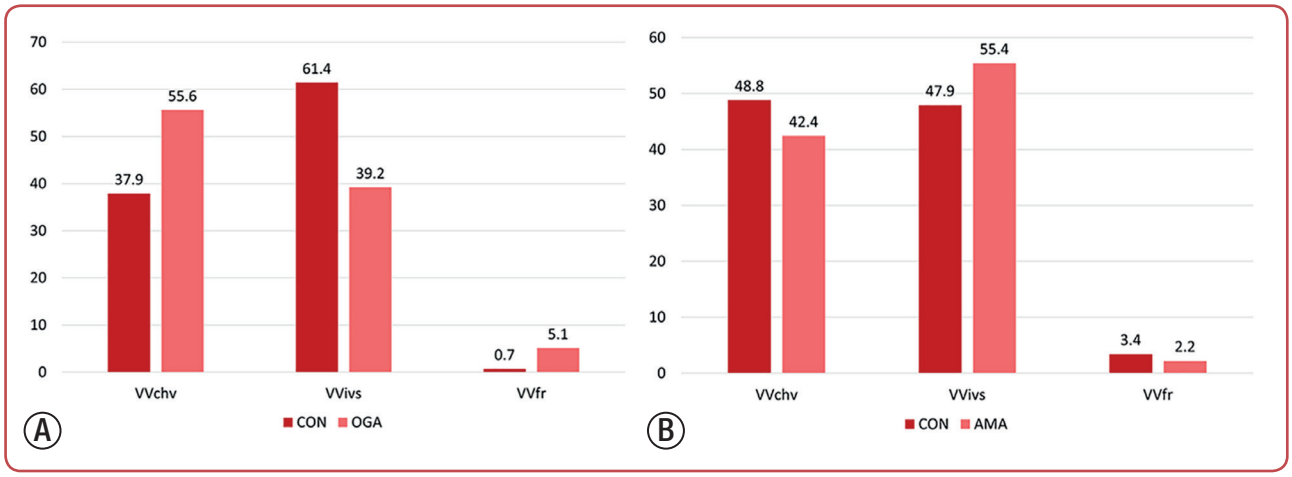

Figure 2: (A) Influence of gestational age on the volume of chorionic villi (VVchv), space between villi (VVivs), placental fibrinoid (VVfr) in control group (CON) and in older gestational age group (OGA). Increased VVchv and VVfr and reduced Vvivs in OGA group, $p < 0,001$, Student t-test. (B) Influence of maternal age on VVchv, Vvivs and VVfr between control group (CON) and advance maternal age group (AMA). A small difference in placental tissue volume between groups, $p = 0.11$, Student t-test.

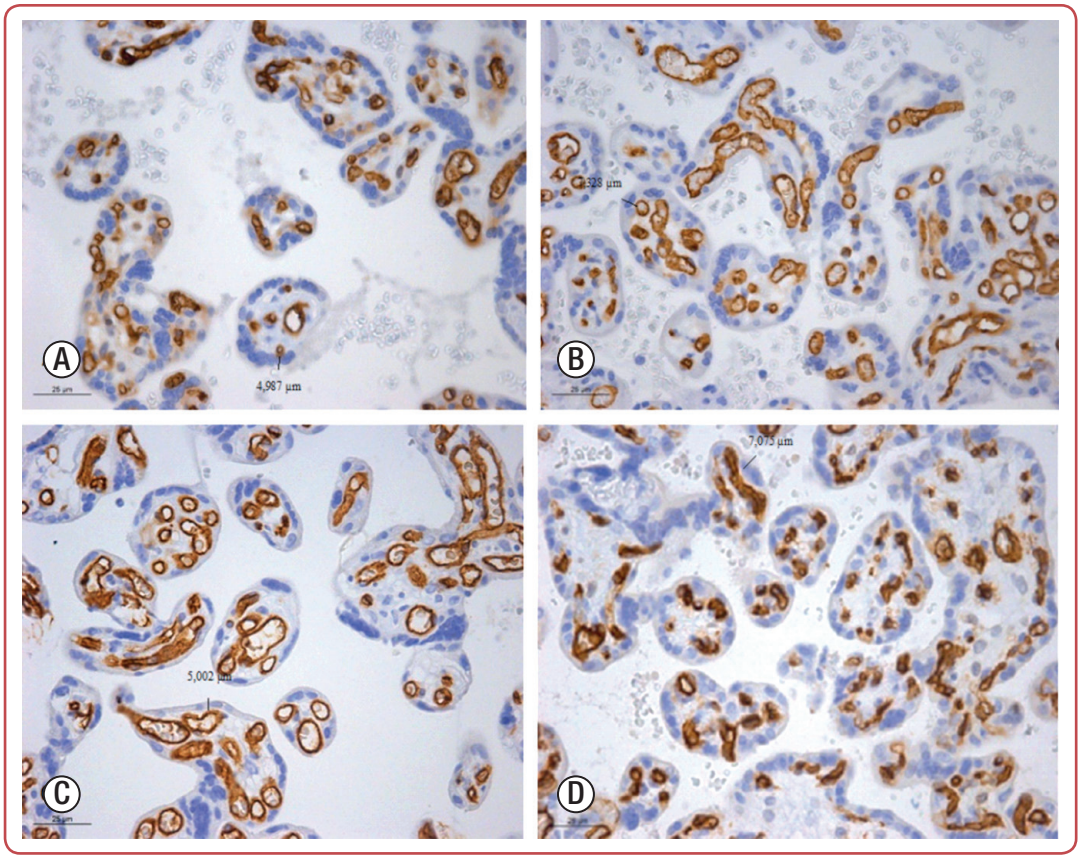

Figure 3: Immunohistochemical analysis of chorionic villi of the placenta. Immunohistochemical staining with CD34 antibodies, magnification 40 x, scale bar 25 µm; A) control group, gestational age of below 38 weeks of pregnancy, B) older gestational age, gestational age of more than 38 weeks of pregnancy, C) control group, maternal age below 35 years, D) advance maternal age. The image shows immunopositive endothelial cells of blood vessels of chorionic villi and components of the placental membrane, increased thickness of placental membrane in the images B and D (scale bar).

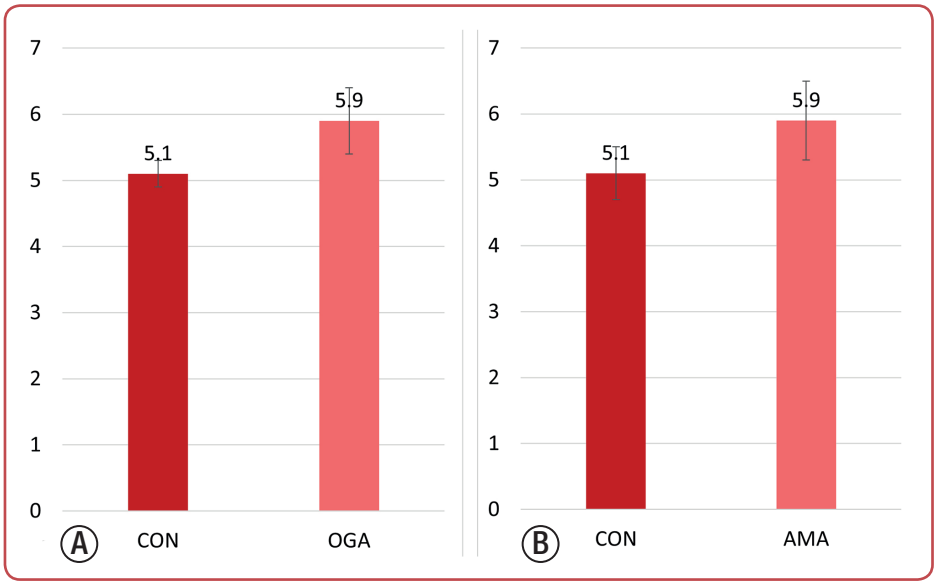

Figure 4: Influence of gestational age on placental membrane thickness represented in µm. A) control group (CON), older gestational age group (OGA), significant increase in the thickness of the placental membrane of placenta OGA group, p = 0.0003, Student t-test. B) Influence of maternal age of control group (CON) and advanced maternal age (AMA) on placental membrane thickness represented in µm, significant increase in the thickness of the placental membrane of AMA placentas p = 0.0003, Student t-test.

Comparing the thickness of the placental membrane in pregnant women of different ages, the values were lower in younger pregnant women, control group and their average value was 5.15 µm, while in the advanced maternal age group, the average value was 5.89 µm, CON: mean ± SD = 5.1 ± 0.4, AMA: mean ± SD = 5.9 ± 0.6. There was a significant statistical difference between these two groups (Figure 3 and 4). However, the thickness of the placental barrier structures was higher and more dependent to the gestational age then to the pregnant woman age.

Discussion

As the interest in using the placenta for research purposes grows, it is necessary to systematise the criteria for their selection. The morphometry as a fairly objective method was used to assess tissue morphology on a histological section. The results of this study clearly showed that with increasing maternal age and gestational age, there is an increase in the volume of villus tissue and thickening of the placental barrier, accompanied by the reduction of intervillous space volume.

The maternal part of the placenta consists of decidua basalis and intervillous space filled with maternal blood, while the foetal part consists of the chorionic plate, composed of amnion, chorion and villi. Placental villi, surrounded by maternal blood, represent the basic transport unit of this temporary organ. All the exchange between the mother and the foetus takes place at the level of the placental membrane, which is made up of trophoblastic cells, the endothelium of the blood vessels inside the villi and the connective tissue that is inserted between them.[15, 16] It is known that the average thickness of the placental membrane is 5 µm. Any change in the structure of this membrane can lead to disorders in the exchange of substances between the mother and the foetus, as well as the fact that the morphology of the placenta is a reflection of the mother's state of health. This statement has been proven by numerous studies in which the influence of chronic non-communicable diseases has been taken into consideration.[13, 17, 18] In addition to the age of the mother, the influence of the gestational age on the volumes of the examined structures was also monitored. Placentas of gestational age from the 36th week to the 41st week are considered mature placentas and in this period of time it is safe to terminate the pregnancy with childbirth. In practice, a certain number of pregnancies of healthy pregnant women ends with childbirth in the period from the 36th to the 38th week of gestation, so it is possible to sample such placentas. The most common indications for childbirth at this gestational age are multiple pregnancies, or in the case when the previous pregnancy ended by Caesarean section and the interval between pregnancies is short. With increasing gestational age, there is an increase in the volume of terminal villi and a decrease in the volume of intervillous space, which shows that a decreased volume of maternal blood is present in the intervillous space, as well as an increase in the foetus's need for nutrition and oxygen due to its growth.

As expected, there was also an increase in fibrinoid volume in placentas of higher gestational age and the recorded difference was statistically significant, which correlates with the findings of other researchers.[19, 20] Marković et al in their research showed that the placentas of adolescent pregnant women have a lower birth weight and decreased volumes of chorionic villi compared to pregnant women who are older than eighteen years. They also showed that although these are healthy pregnant women, these pregnancies are considered risky because the foetuses are born with a lower body weight.[21] When the volumes of chorionic villi and intervillous space in presented examined groups according to the age of the pregnant woman were compared, the difference obtained was not statistically significant. However, results showed that with the increase in gestation and the age of the pregnant woman, there was thickening of the placental membrane. The multiplication of the connective tissue between the endothelial cells and the trophoblast was found and increase in the volume of the trophoblast, thickness of the placental barrier increased to 7.01 µm. There was a small number of studies that dealt with the morphology of human placentas in pregnancies at advanced maternal age. This issue has been mostly studied in animal model. Napso et al found that in Sprague Dawley female rats of older age (6-9 months equivalent to 35 years) the volumes of the placental membrane increased at the expense of the increase in the volume of trophoblasts and the connective tissue between the endothelium and trophoblasts. The multiplication of connective tissue in this area only hinders the exchange of substances between

the mother and the foetus, which also leads to an increase in oxidative stress in the placenta.[8, 22]

Trophoblast cells are key cells for the growth and development of the placenta. Apart from their role in the exchange of gases and nutrients, they have a metabolic and endocrine role and are largely responsible for the secretion of human chorionic gonadotropin.[23] Their activity is mostly influenced by the transforming growth factor-TGF-β, so that any change in trophoblast volume leads to disruption of the dynamics of the cell itself and consequent inhibition of this signalling pathway.[24]

Although the age of the pregnant woman itself did not lead to major changes in the volume of the examined structures, there are still discrete changes at the level of the placental membrane and possible disruptions in its dynamics. Since in the group of pregnant women of advanced maternal age the placentas were of different gestational ages, there was a possibility that the gestational age still has a greater influence on the morphology than the age of the pregnant woman itself, because after all, these were healthy individuals.

Limitations of this study is that is based only on morphometry as a research method. But as stated earlier, it is a fairly objective method to assess tissue morphology on a histological section that does not require use of additional fundings.

Conclusion

The study indicates that both maternal age and gestational age significantly affect placental morphology, with notable increases in the volume of villus tissue and thickening of the placental barrier as these factors rise. However, the changes were more closely associated with gestational age than with maternal age, particularly in healthy pregnancies. Therefore, when selecting placental tissue for regenerative research, only placentas from women under 35 years old and with gestational ages up to 38 weeks should be selected.

Ethics

This research was approved by the Ethical Committee of the Faculty of Medicine of the University of Banja Luka, decision No 18/4.167/21, dated 4 October 2021.

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None.

Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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