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Macromicroscopic anatomy of the placenta after *in vitro* fertilization

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Abstract

Aim – to obtain new data on the macromicroscopic anatomy of the placenta in pregnancy after in vitro fertilization.

Material and methods. The work was performed on 60 placentas after in vitro fertilization. Morphologic study was performed on 30 placentas after IVF. Two fragments were isolated from each placenta – from the marginal and central zone. Serial histotopograms stained according to the Van Gieson method were made. Ultrasound examinations were performed at 20.4-21.1 weeks of gestation. The slice size of the marginal sinus and the area of the placenta were studied.

Results. The median thickness of the choroidal lamina was 250 μm in the central zone and 166.5 μm in the marginal zone; the median vascular diameter was 1653 μm in the central zone and 1040 μm at the edge of the placenta. The median basal lamina thickness was 300 μm in the central zone and 210 μm at the margin. The median length of septa in the central and marginal zones of the placenta was 19893.5 μm and 16007 μm, respectively, and the width of septa was 300 μm in the central zone and 240 μm in the marginal zone. At 20-22 weeks, ultrasound scans can reveal the marginal sinus. The slice shape of the marginal sinus varied from triangular to irregular: the frequency of triangular shape was 40%, arrow-shaped – 30%, irregular shape – 30%.

Conclusion. Thus, it is possible to distinguish three zones in the placenta after IVF at the macromicroscopic level: subchorionic, middle and suprabasal, which have their own histotopographic picture.Quantitative characteristics of placental structures are connected to the place of umbilical cord attachment and have differences in the marginal and central zones.

The shape of the marginal sinus when assessed by ultrasound scanning is different (triangular, arrow-shaped, irregular), with the largest area sizes noted for the irregularly shaped marginal sinus.

Keywords: placenta; macromicroscopic anatomy, *in vitro* fertilization.

Conflict of Interest: nothing to disclose.

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Abbreviations
IFV – *in vitro* fertilization; ART – assisted reproductive technologies.

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Макромикроскопическая анатомия плаценты после экстракорпорального оплодотворения

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Аннотация

Цель – получить новые данные по макромикроскопической анатомии плаценты при беременности после экстракорпорального оплодотворения (ЭКО).

Материал и методы. Работа выполнена на 60 плацентах после ЭКО. Морфологическое исследование проведено на 30 плацентах после ЭКО. Из каждой выделяли два фрагмента – из краевой и центральной зоны. Изготавливались серийные гистотопограммы, окрашенные по методу ван Гизона. Ультразвуковые исследования проводились в сроке беременности 20,4–21,1 недели. Изучались размеры среза краевого синуса и площадь плаценты.

Результаты. Медиана толщины хориальной пластинки составила 250 мкм в центральной зоне и 166,5 мкм в краевой зоне; медиана диаметра сосудов – 1653 мкм в центральной зоне и 1040 мкм у края плаценты. Медиана толщины базальной пластинки составила 300 мкм в центральной зоне и 210 мкм у края. Медиана протяженности септ в центральной и краевой зонах плаценты составила 19893,5 мкм и 16007 мкм соответственно, а ширина септ – 300 мкм в центральной зоне и 240 мкм в краевой зоне. В сроке 20–22 недели при ультразвуковом сканировании можно выявить краевой синус. Форма среза краевого синуса варьировала от треугольной до неправильной: частота встречаемости треугольной формы – 40%, стреловидной формы – 30%, неправильной формы – 30%.

Заключение. В плаценте после ЭКО на макромикроскопическом уровне возможно выделить три зоны: подхориальную, среднюю и надбазальную, которые имеют свою гистотопографическую картину. Количественные характеристики структур плаценты имеют связь с местом прикрепления пуповины и различия в краевой и центральной зонах. Форма краевого синуса при оценке ее методом ультразвукового сканирования различна (треугольная, стреловидная, неправильная), при этом наибольшие размеры площади отмечены для краевого синуса неправильной формы.

Ключевые слова: плацента, макромикроскопическая анатомия, экстракорпоральное оплодотворение.

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Список сокращений
ЭКО – экстракорпоральное оплодотворение; ВРТ – вспомогательная репродуктивная технология.

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INTRODUCTION

Considering the increasing need in the in-vitro fertilization (IVF) and the respective necessity of studying the pregnancy and childbirth after the IVF, the formation of the feto-placental complex in the pregnancy after *in vitro* fertilization remain some of the most important questions. The studies of the placenta structure after the IVF usually consider its histology and pathomorphology [1–4]. It was proven, however, that the changes in the macromicroscopic anatomy of the placenta are instrumental in the development of feto-placental blood circulation disorders and complications of pregnancy (gestational toxicosis and intrauterine growth retardation). Maintenance of such pregnancy and prenatal care are more than a medical problem being important social and psycho-emotional aspects of life of families expecting childbirth against reproductive problems [5–8].

The ultrasound scanning method has made it possible to conduct screening tests on pregnant women, which includes diagnostics of placental development pathology[1]. Currently, antenatal ultrasonic examination is used generally to study such a pathology of the placenta as its growing into the uterine wall [9, 10]. Given that the protocol of the second ultrasonic screening includes descriptions of placenta position in the uterine cavity, measurement of its thickness and study of specifics of its structure, as well as measurement of the blood flow velocity in the umbilical arteries and the uterine arteries [11–13], it is important to elaborate the data on its macromicroscopic anatomy. Starting from the second trimester of pregnancy, it is possible to identify the marginal sinus, so important in the utero-placental blood flow [14].

AIM

To obtain new data on the macromicroscopic anatomy of the placenta in pregnancy after in vitro fertilization.

MATERIAL AND METHODS

The object of the study was 60 placentas after IVF, of which 30 were subjected to morphological examination. Inclusion criteria: urgent delivery in case of singleton pregnancy after IVF, absence of severe extragenital pathology and pregnancy complications in women. Exclusion criteria: premature birth, multiple pregnancy.

To study the macromicroscopic anatomy of the placenta, fragments were isolated from the marginal and central zones. After passing through alcohols of increasing concentration and pouring into celloidin, serial histotopograms were made. The sections were stained using the standard van Gieson method. The histotopograms were studied under the MBS-10 and MicroOptix MX-1150 T microscopes, digital eyepiece camera ToupCam DCM 500 at 8×, 20×, and 30× magnification with photographing of each specimen.

Ultrasonic examinations were performed using the Voluson S10 unit with the RAB6-RS and Samsung HS 70 (A) probes, and with the 5-9 MHz microconvex probe at the gestational age of 20.4–21.1 weeks. The following criteria were studied: longitudinal and transverse dimensions of the cross-section of the marginal sinus, the area of the cross-section of the placenta and the area of the cross-section of the marginal sinus by tracing.

The obtained data were subjected to variational statistical processing on a personal computer (operating system: Windows XP, Microsoft Word Excel 2010 and IBM SPSS Statistics 20.0 software suites). Descriptive statistics of quantitative data were carried out after analyzing them for the nature of distribution by calculating the Kolmogorov-Smirnov criterion. All variation series had a distribution different from normal, due to this the central tendency was described using the median with the diversity of quantitative features using the interquartile range, presented in the work in the Me format [Q_1-Q_3]. The level of statistical significance of differences between the compared groups was determined using the Kruskal-Wallis test. In the statistical analysis procedures, the level of statistical significance (p) was calculated, the critical value of which in the study was 0.05.

The work was carried out at the Department of Human Anatomy of the Orenburg State Medical University, in the departments of the Orenburg Regional Clinical Hospital No. 2. The study was approved by the local ethics committee of the Orenburg State Medical University (protocol dated November 28, 2022, No. 308).

RESULTS

The macromicroscopic structure of the placenta is the chorionic plate, stained red using the van Gieson method. Above it are the blood vessels significantly varying in diameter from 540 μm to 1939.5 μm. From the choroidal plate, large supporting villi extend to the depth of the placental tissue. The cross-section of the villi below the choroidal plate varies in the wide range from 250 μm to 1470 μm. Inside the stem villi, numerous arterial and venous vessels are located. Close to the center of the section, the number of large villi decreases and the variations in their cross-section sizes diminish coming to 70–150 μm. On the surface of the placenta adjacent to the uterine wall, the uneven surface of the placental wall

[1]Order of the Ministry of Health dated October 20, 2020 No. 1130n "On approval of the Procedure for the provision of medical care in the field of obstetrics and gynecology". Available online: https://base.garant.ru/74840123/

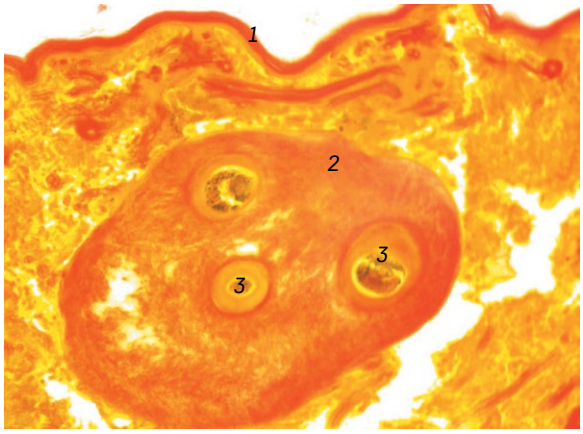

Figure 1. *Transverse section of the villus. Subchorionic zone of the placenta in the central part. Histotopogram. Photos under the microscope MX-1150 (T). Magnification: lens 2.0, eyepiece 10. Van Gieson staining. 1 – choroidal plate; 2 – supporting villi; 3 – villous vessels.*

Рисунок 1. *Поперечный срез ворсины. Подхориальная зона плаценты в центральной части. Гистотопограмма. Фотографии под микроскопом МХ-1150 (Т). Увеличение: объектив 2,0, окуляр10. Окраска по ван Гизону. 1 – хориальная пластинка; 2 – опорная ворсина; 3 – сосуды ворсины.*

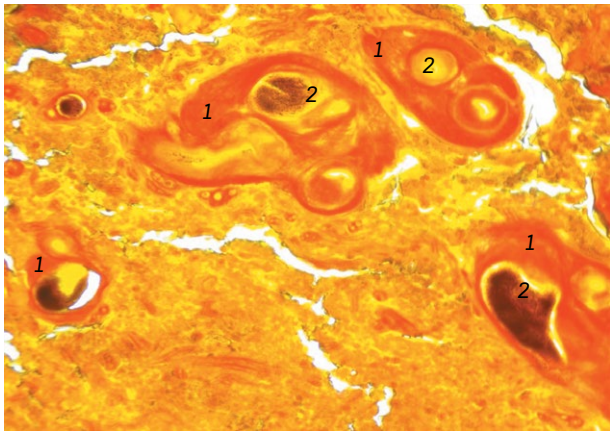

Figure 2. *Group arrangement of villi. Middle zone of the placenta in the central part. Histotopogram. Photos under the microscope MX-1150 (T). Magnification: lens 2.0, eyepiece 10. Van Gieson staining. 1 – intermediate villi; 2 – villous vessels.*

Рисунок 2. *Групповое расположение ворсин. Средняя зона плаценты в центральной части. Гистотопограмма. Фотографии под микроскопом МХ-1150 (Т). Увеличение: объектив 2,0, окуляр 10. Окраска по ван Гизону. 1 – промежуточные ворсины; 2 – сосуды ворсины.*

is clearly detectable, and the boundaries between the two placental cotyledons are seen. These cotyledons are divided by septa. The placental septa are characterized by a manifested polymorphism of shapes and sizes. The depth of their extension to the placental tissue varies from 13350 μm to 30160 μm. Over the basal plate are the terminal villi with smaller cross-sections varying between 45 to 75 μm.

The histotopographic approach towards assessment of the structural elements of the placenta allowed for the identification of three zones in the sections that are different in their macro-microscopic characteristics: the subchorionic, the middle, and the suprabasal zones.

In the subchorionic zone (**Fig. 1**), located below the chorionic plate, large villi with many vessels inside are usually located.

The majority of villi and blood vessels in this area were circular or oval in section. Between the villi of this zone, there were areas where the density of placental tissue was lower.

In the middle zone (**Fig. 2**), the number of intermediate villi and the density of their distribution were grouped. Inside the villi, the blood vessels could be arranged in groups or in pairs. The section of villi and blood vessels was polymorphous, and irregularly shaped structures prevailed.

In the suprabasal zone, located above the basal plate, smaller villi were situated. Their number in this area was lower, and the area of intervillary space was larger.

The macro-microscopy of placenta allows identification of all three zones, there are no stem villi, the number of smaller villi is higher and the area of intervillary space is larger.

The quantitative characteristics of different anatomic structures of the placenta were assessed specifically for placentas with different types of the umbilical cord attachment (**Fig. 3**).

The quantitative characteristic of structural elements of the placenta depending on the umbilical cord attachment location follows in **Table 1**.

In the case of central attachment of the umbilical cord, the quantitative characteristics of the two zones had differences. The medians of all values in the central zone were higher than those for the marginal zone: placenta thickness, by 23.5%; choroidal plate thickness, by 36.4%; diameter of vessels in the choroidal plate, by 75.8%; length of septa, by 27.2%; width of septa, by на 25%; and basal plate thickness, by 42.9%.

In the case of marginal attachment of the umbilical cord, the difference in median values for the similar indicators in the central zone of the placenta were as follows: 28.1% for the placenta thickness; 39.8% for the choroidal plate thickness; 51.5% for the diameter of vessels in the choroidal plate; 25.6% for the length of placental septa; 38.5% for the width of placental septa; 20% for the basal plate thickness.

In the case of sheathed attachment of the umbilical cord, the values tended to be higher in the central zone: 20% for the placenta thickness; 42.9% for the choroidal plate thickness;

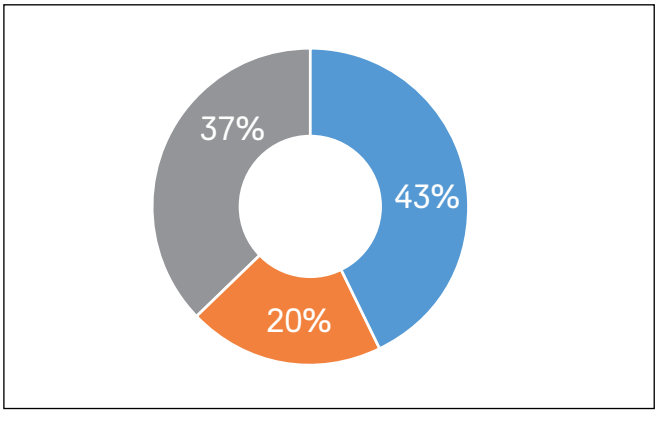

Figure 3. *Options for attaching the umbilical cord to the placenta: central (43%), marginal (20%), sheathed (37%) umbilical cord attachment.*

Рисунок 3. *Варианты прикрепления пуповины к плаценте: центральное (43%), краевое (20%), оболочечное (37%) прикрепление пуповины.*

Parameter		Central attachment	Marginal attachment	Sheathed attachment	P
Central zone					
Placenta thickness, μm	Me	21 000	20 500	24 000	0.458
	$[Q_1-Q_3]$	20 000–25 000	17 750–26 000	20 000–26 000	
Choroidal plate thickness, μm	Me	300	295	303	0.128
	$[Q_1-Q_3]$	245–300	264.8–430.5	250–400	
Diameter of vessels on the choroidal plate, μm	Me	1 600	1 591	1 700	0.458
	$[Q_1-Q_3]$	1 281–1 919.5	1 184.3–1 896.3	1 500–2 151	
Length of septa, μm	Me	20 596	19 725	19 620	0.624
	$[Q_1-Q_3]$	19 365–23 624.5	17 408.5–22 537.8	18 620–25 096	
Width of septa, μm	Me	300	332.5	210	0.163
	$[Q_1-Q_3]$	278–410	200–375	200–364	
Basal plate thickness, μm	Me	300	270	300	0.65
		229–300	200–398.5	212–300	
Marginal zone					
Placenta thickness, μm	Me	17 000	16 000	20 000	0.147
	$[Q_1-Q_3]$	15 000–18 000	14 750–18 500	16 000–25000	
Choroidal plate thickness, μm	Me	220	211	212	0.31
	$[Q_1-Q_3]$	152.5–259	207.5–234	170–300	
Diameter of vessels on the choroidal plate, μm	Me	910	1050	1 212	0.123
	$[Q_1-Q_3]$	750–1 075	920–1 370.8	900–1 400	
Length of septa, μm	Me	16 190	15 700	17 120	0.555
	$[Q_1-Q_3]$	14 737.5–17 742.5	14 247.5–17 435	14 520–20 660	
Width of septa, μm	Me	240	240	300	0.722
	$[Q_1-Q_3]$	200–300	200–355	151–350	
Basal plate thickness, μm	Me	210	225	200	0.835
	$[Q_1-Q_3]$	161–259.5	200–274.5	200–273	

Table 1. *Quantitative characterization of placenta structural elements after IVF depending on the place of umbilical cord attachment*
Таблица 1. *Количественная характеристика структурных элементов плаценты после ЭКО в зависимости от места прикрепления пуповины*

40.3% for the diameter of vessels in the choroidal plate; 14.6% for the length of placental septa, 50% for the basal plate thickness. At the same time, the median width of placental septa was higher in the marginal zone of the placenta by 42.9%.

Thus, the quantitative values for placentas with central and marginal attachment of the umbilical cord have the largest differences between the central and the marginal zones in the vessel diameter in the choroidal plate; in the case of sheathed attachment of the umbilical cord, the largest difference is found in the basal plate thickness.

The comparison of quantitative characteristics of placental elements in the central zone with different types of umbilical cord attachment revealed the following: the median values of the placenta thickness, choroidal and basal plate thickness, diameter of the vessels of the choroidal plate tended to decrease from the central attachment group towards the marginal attachment, and to increase from the marginal attachment group to the sheathed attachment group.

The median length values of placental septa decreased from the group with central attachment to the group with marginal attachment, and from the group with marginal attachment to the group with sheathed attachment.

The median width of placental septa increased from the group with central attachment to the group with marginal attachment, and decreased from the group with marginal attachment to the group with sheathed attachment.

In the case of the marginal zone of the placenta, similar differences were noticed for the median values of placenta

Parameter		Triangular shape	Swept shape	Irregular shape	p
Longitudinal size of marginal sinus section, cm	Me	1.6	1.7	2.7	0.248
	$[Q_1-Q_3]$	1.3–2.0	1.3–2.2	1.3–3.8	
Transversal size of marginal sinus section, cm	Me	0.8	1.0	1.2	0.298
	$[Q_1-Q_3]$	0.6–1.0	0.8–1.4	0.7–1.7	
Area of marginal sinus section, cm^2	Me	0.7	1.1	1.9	0.147
	$[Q_1-Q_3]$	0.4–1.0	0.7–1.3	0.5–3.0	
Area of placenta cross- section, cm^2	Me	33.2	29.5	32.3	0.147
		28.6–35.7	26.3–37.4	29.8–38.4	

Table 2. *Quantitative characterization of the size of the marginal sinus of the placenta*
Таблица 2. *Количественная характеристика размеров краевого синуса плаценты*

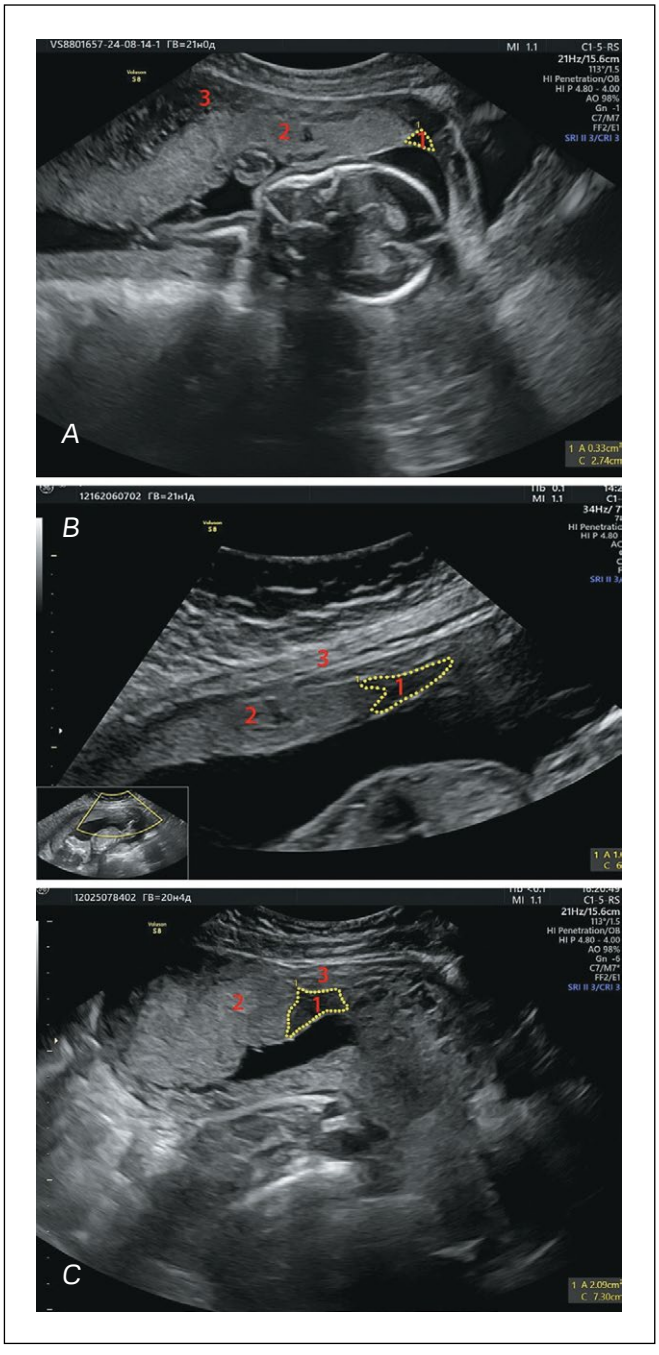

Figure 4. *Various shapes of the edge sine slice. Ultrasound scan of the placenta in the B-mode. A – triangular, gestational age 20 weeks 6 days; B – swept, gestational age 21 weeks 1 day, C – incorrect, gestational age 20 weeks 4 days. 1 – marginal sinus; 2 – placenta; 3 – uterine wall.*

Рисунок 4. *Различные формы среза краевого синуса. Ультразвуковая сканограмма плаценты в В-режиме. А – треугольный, гестационный возраст 20 недель 6 дней; В – стреловидный, гестационный возраст 21 неделя 1 день, С – неправильный, гестационный возраст 20 недель 4 дня. 1 – краевой синус; 2 – плацента; 3 – стенка матки.*

thickness and choroidal plate thickness, diameter of the vessels of the choroidal plate and the length of placental septa; at the same time, the median width of placental septa was similar in the groups with central and marginal attachment of the umbilical cord, yet the same value increased from the marginal attachment to the group with sheathed attachment. The median value of basal plate thickness increased from the central attachment group to the marginal attachment group, and decreased from the marginal attachment group to the sheathed attachment group.

In addition, ultrasonic scanning allows for the description of the marginal sinus at the gestational age of 20–22 weeks; it is located on the periphery of the placenta and limits the intervillary space.

In the ultrasonic scans, the marginal sinus is shown as a space of varied shaped. The section form of the marginal sinus is closer to triangular (**Fig. 4A**) in 40% of the cases; the swept (**Fig. 4B**) and irregular (**Fig. 4C**) shapes being registered in 30% of the cases each, respectively (**Fig. 4**).

The quantitative characteristics of the dimensions of the marginal sinus follow in **Table 2**.

It is seen from the table that the largest longitudinal size is characteristic for the irregular-shaped marginal sinus, and the smallest, for the triangular-shaped. At the same time, it increases by 5.9 and 37% from the triangular-shaped towards irregular shaped marginal sinus, respectively. The transversal size of the swept-shaped marginal sinus for all studied shapes increases from the triangular towards irregular shape by 20% and 20%, respectively. The difference in the area between the triangular and irregular shapes of marginal sinus is 2.7 times.

All the quantitative values of the irregular-shaped marginal sinus prevail in the triangular and swept-shaped forms.

DISCUSSION

According to the literature [15, 16], the fetal surface of the placenta is smooth and glossy; it is covered with the amniotic sac, below which branched blood vessels of various diameter are clearly seen. This confirms the data of this study to the effect that the diameter of vessels of the choroidal plate is highly variable. The intervillary space on the fetal side is formed by the choroidal plate and villi attached to it, and on the maternal side, it is restricted by the basal plate, decidual membrane and partitions (septa) branching from it. It is found that the septa are of varying shape and length, which, in its turn, depends on the location of the umbilical cord location, and on the zone of placenta (central or marginal).

The structure of the fetal part of the placenta is represented by numerous chorionic villi, which are united into structural formations, i.e. cotyledons.

According to A.P. Milovanov and S.V. Savelieva (2006) [17], the villous tree presents three levels of branching. Supporting villi of levels 1, 2, and 3 form the fetal part of the placenta, i.e. they are found in the subchorionic zone and comprise the anatomical framework of the cotyledon. The intermediate differentiated villi branch from Level 2 and 3 supporting villi and range in sizes from 75 to 150 μm, i.e. are found in the middle zone of the placenta. The terminal villi branch from intermediate villi, their diameter is 40–80 μm. This data found its confirmation in the findings of this study.

Foreign researchers [18–20] note that placenta previa is detected more often after the IVF, as is, respectively, marginal and sheathed attachment of the umbilical cord; however, differences in the macro-microscopic anatomy are minor and statistically insignificant as compared to placentas after in vivo fertilization.

According to M.V. Medvedev (2016) [14], the ultrasonic scanning allows the detection of the marginal sinus, starting from the second trimester. It lies on the periphery of the placenta and has the exterior of an uneven slit sized from 0.5 to 1.5 cm. The same is supported by this study; besides, the latter

identified the shapes of the marginal sinus (triangular, swept, and irregular) and describes variations in its size depending on the shape.

The fundamental research in the 'mother-placenta-fetus' system in the post-IVF pregnancies are quite prospective for a variety of reasons. Firstly, the state policy focuses on tackling the demographic situation and entails increased financing of the number of IVF cycles. Secondly, the 'mother-placenta-fetus' system has its proper morphological and clinical peculiarities in the pregnancies achieved by assisted reproductive technologies and requires special attention from the obstetricians-gynecologists and neonatology physicians in terms of maternity and neonatal care. Thirdly, numerous studies have found that there is a regional component both in the fetometry and in the morphology of placenta in the normal conditions (without assisted reproductive technologies), therefore, a promising line of research is the development of the 'morphological profile' of the 'mother-placenta-fetus' system for women after IVF in various administrative units of the Russian Federation.

CONCLUSION

It is possible to identify three zones in the placenta after the IFV: the subchorionic, middle and suprabasal zones, each with a histotopography of its own.

The quantitative characteristics of the placental structures are related to the location of the umbilical cord attachment, and differences in the marginal and central zones.

The shape of the marginal sinus, as inspected by ultrasonic scanning, is different (triangular, swept, and irregular), the largest area sizes detected in the irregularly shaped marginal sinus.

ADDITIONAL INFORMATION	ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ
Ethical review. The study was approved by the local ethics committee of Orenburg State Medical University (protocol dated 28.11.2022, No. 308).	***Этическая экспертиза.*** Исследование одобрено локальным этическим комитетом ОрГМУ (протокол от 28.11.2022 года №308).
Study funding. The study was the authors' initiative without external funding.	***Источник финансирования.*** Работа выполнена по инициативе авторов без привлечения финансирования.
Conflict of interest. The authors declare that there are no obvious or potential conflicts of interest associated with the content of this article.	***Конфликт интересов.*** Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с содержанием настоящей статьи.
Contribution of individual authors. I.V. Mitrofanova: concept and design of the study, collection, analysis and interpretation of data, preparation of the text. E.D. Lutsay: concept and design of the study; editing of the manuscript. All authors gave their final approval of the manuscript for submission, and agreed to be accountable for all aspects of the work, implying proper study and resolution of issues related to the accuracy or integrity of any part of the work.	***Участие авторов.*** И.В. Митрофанова – концепция и дизайн исследования, получение, анализ и интерпретация данных, подготовка текста. Е.Д. Луцай – концепция и дизайн исследования; редактирование рукописи. Все авторы одобрили финальную версию статьи перед публикацией, выразили согласие нести ответственность за все аспекты работы, подразумевающую надлежащее изучение и решение вопросов, связанных с точностью или добросовестностью любой части работы.

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