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CHORIONIC VILLI OF HUMAN EMBRYOS, DIED OUT OF SPORADIC AND RECURRENT MISCARRIAGES: A LECTIN HISTOCHEMISTRY ANALYSIS

9 tissue samples of chorionic villi of human embryos, died in the first trimester of pregnancy at 4-13 weeks in-utero were investigated. Two research groups were formed: the first - sporadic miscarriages, the second - recurrent miscarriages. Control group consisted of 5 chorionic villi tissue samples of human embryos obtained after artificial abortion of physiological pregnancies at respective gestational age accomplished due to women's demand. Histological material was fixed in 4% neutral formalin solution. To study the general morphology the 5-7 μ m thick sections were stained with hematoxylin and eosin. Hydrocarbon cell surface glycoproteins determinants of chorionic villi and their intracellular compartments were studied by lectin-peroxidase technique using lectins PNA, GNA, WGA and PFA. Viewing and photographing of histological samples was performed using a microscope "Swift Instruments International", equipped with camera "Echo-Imager 502 000" using computer program "TopView 3.2".

General morphological studies shown changes of the shape and structure of chorionic villi in the investigated samples, compared with the control. Based on lectin histochemical studies the different exhibition level of lectin receptors in the chorionic villi structural components of 4-13-week-old human embryos was observed. The highest expression of lectin receptors WGA, GNA and PFA detected in the study group of recurrent miscarriage, indicating a violation of biopolymers end-stage glycosylation due to excessive activation of glycosidases, compared with a group of sporadic miscarriage and control group.

Key words: chorionic villi, sporadic miscarriage, recurrent miscarriage, lectin histochemistry, human embryos.

The investigation was carried out as a fragment of SRW "Lectin histochemistry of carbohydrate determinants in normal and pathologically changed cells and tissues"

The most common complication of pregnancy is its miscarriage. The term "pregnancy miscarriage" (PM) includes spontaneous abortion in between fertilization and 16th-26th weeks of fetal development, the last term variations depending on the particular country medical legislation rules [8, 14]. About 15% of all pregnancies terminate with abortions, out of which about 3/4 occur during the first trimester [16]. The most frequently described risk factors of PM are woman's age and pathology of the fetus. In particular, women 30 years of age possess risk for PM as 1:6, aged 30-40 – 1:5, after 40 – 1:4 [15]. Regarding fetal pathology, life incompatible disorders in 50% of cases are caused by chromosomal abnormalities [6]. In general, about 25% of women at least once during their life lost pregnancy [15, 19].

Beside sporadic pregnancy loss, or sporadic miscarriage (SM), there also exists a recurrent miscarriage (RM) – regular (three or more times) abortion. RM occurs in 1% of all women of fertile age [19, 21]. The probability of having a child for women with RM is calculated as 60%, while for women with SM this ratio is 80% [21]. The literature describes a wide number of possible RM preconditions, the most common of which are as follows: antiphospholipid syndrome in women, fetal or parental genetic abnormalities, anatomical and endocrine factors, inherited thrombophilia and immune factors [17, 21]. In this range the immune factors are predominating, especially at early (before 14 weeks) pregnancy loss. In particular, 45% of early pregnancy loss is accompanied by a parental intolerance and defined by the term "immune infertility" [9, 16]. Under these conditions, fetal cells are recognized by the maternal immune system as "foreigners", similarly to bacteria, viruses or tumor cells, and mother's immune response is directed towards their elimination [13]. With this regard investigations of mechanisms leading to incorrect interaction between fetal cells and maternal immune system is essential for understanding of its consequence – pregnancy miscarriage.

One of the main receptor systems in any cell (including fetal) is the cover layer of glycoproteins and glycolipids on the surface of plasma membrane [3, 11]. Namely, carbohydrate determinants of surface glycoconjugates play a key role in the recognition of fetal cells by the maternal immune system [4, 7]. Subsequently, abnormal changes in these complexes can trigger "wrong" reactivity of maternal immune system directed against the fetus. Lectins – biological recognition molecules, that specifically bind sugar moieties of cell surface and cytoplasmic glycoconjugates [10]. Currently they are widely used in histochemistry and diagnostic histopathology as a tool for studying modifications of cell and tissue carbohydrate determinants under various physiological and pathological conditions [5, 20].

Aim. The aim of present investigation was to study carbohydrate profiles of chorionic villi glycoconjugates in recurrent and sporadic miscarriages in comparizon to those from normal human embryos obtained after artificial abortions.

Materials and methods. Human embryos. Obtained material included 9 human embryos with chorionic villi tissue, lost in the first trimester of pregnancy (4-13-weeks gestational age), out of which 6

referred to sporadic, and 3 – to recurrent miscarriages. Control group specimens consisted of 5 embryos with chorionic villi tissue of corresponding gestational age obtained after artificial abortion of physiological pregnancies accomplished due to women's demand (Table 1). Research performed in compliance with the main provisions of GCP (1996), the European Convention on Human Rights and Biomedicine on 04.04.1997 and the Helsinki Declaration of the World Medical Association on ethical principles of scientific medical research involving human subjects (1964-2008) and MOH Ukraine order №690 on 23.09.2009, as evidenced by the conclusion of biomedical ethics commission LNMU (protocol number 6 from 24.06.2013).

Table 1

Quantitative and gestational characteristics of the embryonic material

Gestational age of the embryo (weeks)	Group of sporadic miscarriage (SM); number of samples	Group of recurrent miscarriage (RM); number of samples	Control group (CG); number of samples
4-5	-	1	1
6-7	2	1	2
7-9	-	2	2
11-13	1	1	-

CV tissue samples.

Human embryos of the lost pregnancies and artificial abortions were three times washed in 0,9% NaCl solution. Chorionic villi were removed under a microscope, fixed in 4% neutral formalin and embedded in paraffin wax according to the standart protocol [25]. For general morphology sections 5-7 μ m thick were stained with haematoxylin and eosin. Glycoepitopes of chorionic villi glycoconjugates were detected by a set of 4 lectins, presented in Table 2, using lectin-peroxidase technique as described elsewhere [10]. In particular, deparaffinated sections were incubated for 45 min at room temperature with lectin- peroxidase conjugate (concentration of 10-25 μ g / ml) in buffered saline, pH 7.4; visualization of lectin receptors was performed with the use of 0.05% solution diaminobenzidine tetrahydrochloride (Sigma, St. Louis, MO, USA) with the addition of 0.015% H₂O₂ [25].

The control of lectin binding reaction was performed by removing the lectin from the protocol, as well as the exclusion of inhibition reaction of endogenous peroxidase with methanol [24]. The lectins were gifted by Dr. W. Antonyuk "Lectinotest laboratory" (Lviv, Ukraine)

Table 2

Characteristics of lectin panel *

Lectin abbreviation and source	Carbohydrate specificity	Complementary oligosaccharide residue
PNA, Peanut agglutinin	DGal	DGal(β 1-3)GalNAc
WGA, Wheat germ agglutinin	DGlcNAc>NeuNAc	NeuNAc(α 2-6)Gal(β 1-4)GlcNAc, Man(β 1-4) GlcNAc(β 1-4)GlcNAc
GNA, Galanthus nivalis agglutinin	α DMan	Man(α 1-3)Man, oligomannoside N-glycans
PFA, Perca fluviatilis agglutinin	α LFuc	Fuc(β 1-2)Gal(β 1-3)GlcNAc

* Carbohydrate specificity of the used lectins according to [14,24].

Viewing and photographing of histological samples was performed using a microscope "Swift Instruments International", equipped with camera "Echuo-Imager 502 000" using computer program "TopView 3.2".

Results and its discussion. Evaluation of chorionic villi tissue, painted with hematoxylin and eosin showed that at the studied stages of development chorionic villi formed following structural components: syncytiotrophoblast (STB), cytotrophoblast (CTB), mesenchyme (MC), hemocapillaries and Hofbauer cells (HC) (fig. 1). Experimental samples (sporadic and recurrent abortions) have destructive changes in the morphology of the structural components that occur in irregularly shaped villi and mesenchymal exfoliation and edema, which was not observed in control models derived from artificial abortions.

To evaluate the results of all samples were grouped according to the following groups: research, a group of sporadic abortions (SA) and a group recurrent abortions (RA) and in four age categories: 4-5 - weeks, 6-7 - weeks, 7-9 - weeks and 11-13 - weekly embryos. The affinity of lectins to the cells evaluated in 6-point scale, the results are presented in Table 3 and Figures 2-5.

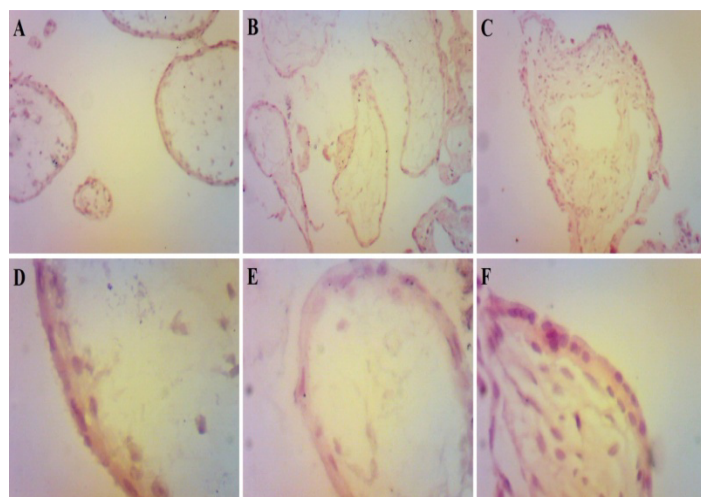


Figure 1. Chorionic villi, painted with hematoxylin and eosin (A, D - control samples, increasing x300 and x600, respectively; B, E - samples obtained during spontaneous abortion, increasing x300 and x600, respectively; C, F - samples received from recurrent miscarriage, increasing x300 and x600, respectively).

Generally, lectin histochemical studies have shown high affinity of selected lectins to cells syncytiotrophoblast (STB), cytotrophoblast (CTB), Hofbauer cells (HC) and the mesenchyme cells (MC) in all experimental as well as control models.

In the control group (embryos obtained from artificial abortions) the highest affinity for the selected lectins was observed in STB cells. As shown in Table 3, the highest affinity (5 points from 6-point rating system) to the cells STB cells lectin WGA, and the lowest (from 0-1 to 3 points out of possible 6-) - GNA. Average level of affinity (1 to 4 points) had lectin PFA (Table 3), and high (3 to 5 points) - lectin PNA (Table 3).

Table 3

Exposure of GNA, WGA, PFA and PNA lectin receptors in chorionic villi structural components of 4-13 - week human embryos in normal and spontaneous abortion in points

Research group	Gestational age (in weeks)	Chorionic villi structural components															
		STB				CTB				HC				MC			
		GNA	WGA	PFA	PNA	GNA	WGA	PFA	PNA	GNA	WGA	PFA	PNA	GNA	WGA	PFA	PNA
Control	4-5	1*	5	1	5	1	4	1	4	1	4	2	4	1	3	1	1
	6-7	3	5	2	3	3	4	2	3	3	4	3	3	2	3	1	3
	7-9	2	5	4	5	2	5	4	4	2	5	3	4	1	3	1	2
	11-13	n/s**															
Sporadic abortions	4-5	n/s															
	6-7	6	6	4	3	6	5	4	3	2	5	1	3	3	4	1	3
	7-9	n/s															
	11-13	3	5	3	3	3	5	2	3	3	4	3	3	2	4	2	3
Recurrent abortions	4-5	5	5	3	3	5	4	2	3	4	4	1	4	3	3	1	2
	6-7	6	6	4	3	5	5	3	3	5	6	3	3	5	5	3	2
	7-9	5	5	4	3	5	5	3	3	5	4	3	3	4	3	3	2
	11-13	6	5	5	3	6	5	4	3	5	4	2	2	5	4	4	1

Note: * - level of lectin receptors expression in chorionic villi cell components in 6- point scale; ** - has not been studied.

Cytotrophoblast cells and Hofbauer cells showed almost the same affinity for the selected lectins. Thus, the highest (4-5 points in the 6-point scale) parameters was observed in WGA lectin (Table 3), and the lowest (from 0-1 to 3 points) in GNA (Table 3). Lectins PNA and PFA revealed almost identical intermediate level affinity (Table 3) to the CTB cells and HC. Results of lectin histochemical studies showed slightly lower (compared with other cells) affinity of selected lectins to the mesenchyme cells. As shown in Table 3, the level of affinity for these cells ranged from very low (0-1 points) in the PFA lectin to low (up to 2 points) - in GNA and PNA lectins and medium (3-points) - in WGA.

Similar to the above described control group, in SA group highest affinity for selected lectins distinguished a STB. As shown in Table 3, the level of affinity has not lowered below 3 points. The highest affinity to STB cells had WGA and GNA lectins, she reached in some cases the maximum 6-scoring point (Table 3). The relatively high affinity for studied lectins were observed in cytotrophoblast cells with the highest level in WGA-lectin (5 point affinity) and GNA-lectin (affinity reached 3 to 6 points). Slightly lower level of affinity was observed in mesenchymal cells and Hofbauer cells: 1-3 points in PFA-lectin, 2-3 points in PNA- and GNA-lectins and 4-5 points in WGA.

In the experimental group of RA, investigated chorionic villi cells were characterized by high affinity for all investigated lectins. Highest affinity for lectins, as in the case of previously described groups, observed in STB cells. As shown in Table 3, it reaches the highest level of affinity in lectins GNA and WGA, above average (3 to 5 points) - in the PFA-lectin (Table 3) and the average 3-point level in lectin PNA (Table 3). Traditionally lower, but also high affinity recorded in CTB cells with the highest 4-6-point level of affinity in GNA and WGA lectins (Table 3). Middle (3-4 points) degree of affinity observed in this experimental group of CTB cells in lectins PNA and PFA (Table 3).

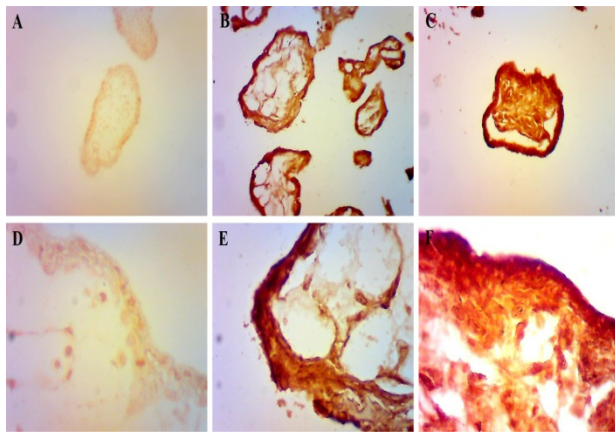


Figure 2. Exposure of GNA lectin receptors in chorionic villi structural components on 6-7 weeks of fetal development (A, D - moderate level of exposure of lectin receptors in all cellular components (control samples) increase x300 and x600, respectively; B, E - overly intense exposure of GNA lectin receptors in the superficial structures (samples obtained during spontaneous abortion), increase x300 and x600, respectively; C, F - overly intense exposure of lectin receptors on the surface and intense in other cellular components (samples obtained from recurrent miscarriage), increase x300 and x600, respectively).

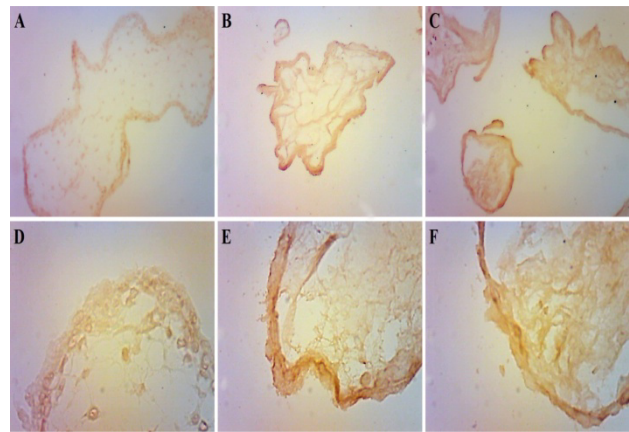


Figure 3. Exposure of PFA lectin receptors in cells of the chorionic villi on 6-7 weeks of fetal development (A, D - moderate level of exposure of lectin receptors on the surface structures, low in Hofbauer cells and almost absent in the mesenchyme (control samples), increase x300 and x600, respectively; B, E - (slightly more expressed than in control samples) exhibiting of lectin receptors in syncytiotrophoblast and locally intense in the basal membrane, moderate in the mesenchyme (samples obtained during spontaneous abortion), increase x300 and x600, respectively; C, F - moderate exposure of lectin receptors in syncytiotrophoblast and mesenchyme (samples obtained from recurrent miscarriage), increase x300 and x600, respectively).

The lowest affinity for selected lectins in RA group had mesenchymal cells with levels of affinity: 1-2 points - for lectin PNA (Table 3), in the range of 1-4 points - for lectin PFA (Table 4), 3-5 points - for GNA and WGA lectins (Table 3).

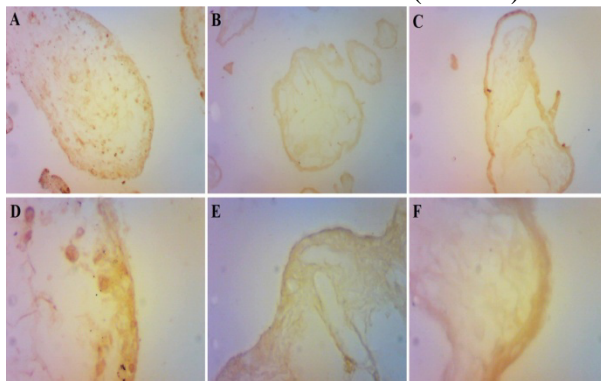


Figure 4. Exposure of PNA lectin receptors in chorionic villi structural components on 6-7 weeks of fetal development (A, D - moderate level of exposure of lectin receptors in all structural components with slightly more pronounced in Hofbauer cells (control samples), increase x300 and x600 respectively; B, E - moderate level of exposure of lectin receptors in all structural components (samples obtained during spontaneous abortion), increase x300 and x600, respectively; C, F - moderate level of exposure of lectin receptors in all structural components of chorionic villi (samples obtained from recurrent miscarriage), increase x300 and x600, respectively).

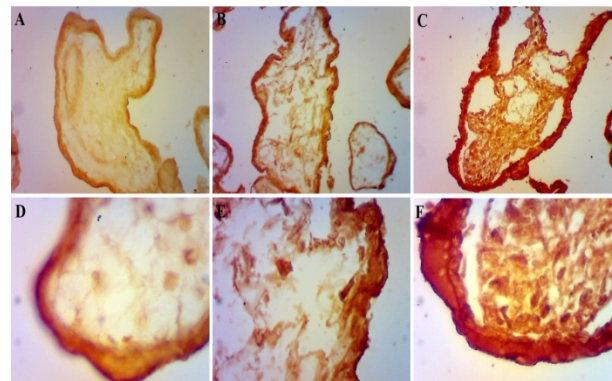


Figure 5. Exposure WGA lectin receptors in chorionic villi cells on 6-7 weeks of fetal development (A, D - a high level of exposition of lectin receptors in syncytiotrophoblast cells and moderate in all other structural components with a slightly more pronounced in cytotrophoblast and Hofbauer cells (control samples), increase x300 and x600, respectively; B, E - very high and high levels of exposure of lectin receptors in the superficial structures and high and moderate in all other structures (samples obtained during spontaneous abortion), increase x300 and x600, respectively; C, F - a high level of expression in all structural components, slightly more pronounced in syncytiotrophoblasts and Hofbauer cells (samples obtained from recurrent miscarriage), increase x300 and x600, respectively).

The next step of our study was a comparison between the results obtained in the different study groups of embryos of the same gestational age. To do this we selected a group of 6-7-week-old embryos, because as, the table shows, it is represented among artificially interrupted pregnancies (control) and sporadically interrupted exactly the same as in the group of recurrent miscarriages. The results are presented in Table 4 and Diagram 1. Apparently, the highest degree of affinity for lectins is observed in experimental group of RA. The level of affinity in this group reaches above average (3-6 points) values with the highest affinity (5-6 points) to WGA- and GNA - lectins.

Group of 6-7 week sporadic abortions, showed slightly lower compared to the RA tropism for the studied lectins, with the level outside of 1 to 6 points. In particular, the lectin PFA showed lowest of affinity with 1 to 4 points, for PNA-lectin - average 3-point values, and WGA- and GNA-lectin characterized by medium and high (from 2 to 6 points) tropism for SA chorionic villi cells.

Table 4

Exposure of PFA GNA WGA PNA lectin receptors in chorionic villi structural components of 6-7 - week human embryos in the control group and the group of spontaneous abortions

Lectins and their carbohydrate peculiarity	Research group	Chorionic villi structural components			
		STB	CTB	HC	MC
PNA (Arachis hypogaea L) β DGal	Control	3*	3	3	3
	Sporadic abortions	3	3	3	3
	Recurrent abortions	3	3	3	2
GNA (Galanthus nivalis L) α DMan	Control	3	3	3	2
	Sporadic abortions	6	6	2	3
	Recurrent abortions	6	5	5	5
WGA (Triticum vulgare L) (DGlcNAc>NeuNAc)	Control	5	4	4	3
	Sporadic abortions	6	5	5	4
	Recurrent abortions	6	5	5	5
PFA (Perca fluviatilis L) α LFuc	Control	2	2	3	1
	Sporadic abortions	4	4	1	1
	Recurrent abortions	4	3	3	3

Note: * - the level of expression of lectin receptors in chorionic villi cell components

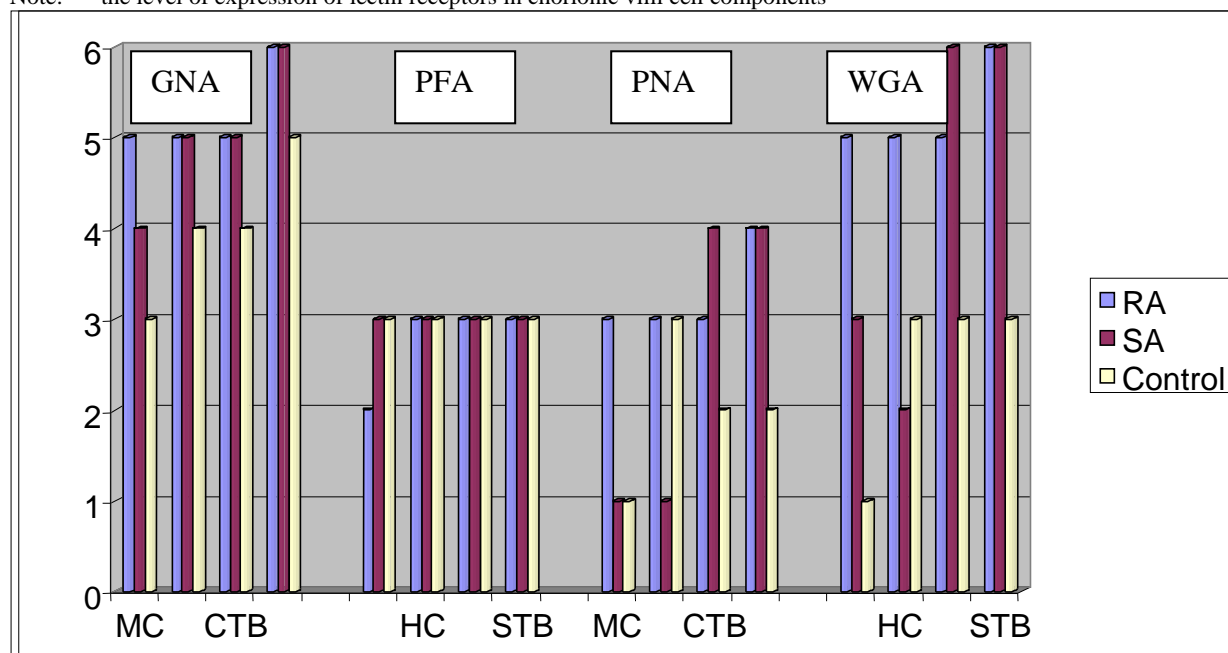


Diagram 1. The intensity of lectin receptors expression in structural components of chorionic villi obtained after artificial abortions, spontaneous and recurrent miscarriages (from left to right - lectins GNA, PFA, PNA and WGA) was assessed by a 6-point scale.

The lowest tropism for the studied lectins characterized the control group of embryos. In particular, the degree of relationship chorionic villi cells of 6-7-week-old artificially aborted embryos described range from 1 to 5 with the lowest rates for lectins PFA, GNA (1-3 points), medium 3-point for PNA-lectin and above average (3 to 5) for WGA-lectin.

Conclusions

1. Microscopic evaluation of histological samples of chorionic villi tissue 4-13-week-old human embryos eliminated artificially or spontaneously helps to evaluate their viability and ability to perform their physiological functions.
2. Lectin histochemical studies showed different intensity of exposition (or different affinity) of lectins: PNA, GNA, WGA and PFA to the chorionic villi tissue of 4-13-week-old human embryos.
3. The highest expression of lectin receptors WGA, GNA and PFA were found in the study group of recurrent abortion indicating a violation of biopolymers end-stage glycosylation due to excessive activation of glycosidases, compared to the sporadic abortion group and the control group of artificially eliminated embryos.
4. Among the studied lectins highest tropism for chorionic villi tissue of human embryos possessed DGlcNAc> NeuNAc-specific lectin WGA, especially to the STB cells.
5. PNA-lectin showed no diagnostic value because its expression levels did not differ between the control and experimental groups, as between the different cell types of chorionic villi.

6. Modification of lectin receptors of chorionic villi structural components in spontaneous and recurrent abortions probably lead to incorrect recognition of these cells by the immune system of the mother, followed by the maternal immune response and, accordingly, the death of the fetus.

Prospects for further research. In future it is planned to study carbohydrate determinants of chorionic villi structural components glycopolymers at the first trimester of pregnancy, using a wider range of lectins with different carbohydrate specificity and immunohistochemical study of chorionic villi using monoclonal antibodies.

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Реферати

ВОРСИНКИ ХОРІОНА ЕМБРІОНІВ ЛЮДИНИ, ЩО ЗАВМЕРЛИ ВНАСЛІДОК СПОРАДИЧНОГО І ЗВИЧНОГО НЕВИНОШУВАННЯ: ЛЕКТИНОГІСТОХІМІЙНИЙ АНАЛІЗ

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Досліджено 9 зразків тканин ворсинок хоріона ембріонів людини, які завмерли у першому триместрі вагітності на 4-13-у тижні внутрішньоутробного розвитку. Сформовано 2-і дослідні групи: перша – спорадичні викидні, друга – звичні викидні. Контрольну групу склали 5 зразків тканин ворсинок хоріона ембріонів людини, отриманих після проведення штучних абортів за бажанням жінки відповідного терміну гестації. Гістологічний матеріал фіксували в 4% нейтральному розчині формаліну. Для

ВОРСИНКИ ХОРИОНА ЭМБРИОНОВ ЧЕЛОВЕКА, ЗАМЕРШИЕ В РЕЗУЛЬТАТЕ СПОРАДИЧЕСКОГО И ПРИВЫЧНОГО НЕВЫНАШИВАНИЯ: ЛЕКТИНОГИСТОХИМИЧЕСКИЙ АНАЛИЗ

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Исследовано 9 образцов тканей ворсинок хориона эмбрионов человека, которые замерли в первом триместре беременности на 4-13-й неделе внутриутробного развития. Сформировано две исследовательские группы: первая – спорадические выкидыши, вторая – привычные выкидыши. Контрольную группу составили 5 образцов тканей ворсинок хориона эмбрионов человека, полученных после проведения искусственных абортов по желанию женщин соответствующего срока гестации. Гистологический материал фиксировали в 4% нейтральном растворе

вивчення загальної морфології зрізи, товщиною 5-7 мкм фарбували гематоксилином та еозином. Вуглеводневі детермінанти глікополімерів поверхні клітин ворсинок хоріона та їх внутрішньоклітинних компартментів вивчали методом лектин-пероксидазної техніки із використанням лектинів PNA, GNA, WGA та PFA. Огляд і фотографування гістологічних зразків проводились із використанням мікроскопа "Swift Instruments International", обладнаного камерою "Echoo-Imager 502 000", за допомогою комп'ютерної програми "TopView 3.2".

Загальноморфологічні дослідження показали зміну форми і структури ворсинок хоріона у досліджуваних зразках, у порівнянні із контрольними. На основі лектиногістохімічних досліджень виявили різне експонування рецепторів лектинів у структурних компонентах ворсинок хоріона 4-13-тижневих ембріонів людини. Найвища експресія рецепторів лектинів WGA, GNA та PFA виявлена у досліджуваній групі звиклого невиношування вагітності, що вказує на порушення кінцевих етапів гліколізування біополімерів внаслідок надмірної активації глікозидаз у порівнянні з групою спорадичних викиднів та контрольною групою.

Ключові слова: ворсинки хоріона, спорадичне невиношування, звичне не виношування, лектиногістохімія, ембріони людини.

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формалина. Для изучения общей морфологии срезы толщиной 5-7 мкм окрашивали гематоксилином и еозином. Углеводородные детерминанты поверхности клеток ворсинок хоріона и их внутриклеточных компартментов изучали методом лектин-пероксидазной техники с использованием лектинов лектинів PNA, GNA, WGA та PFA. Осмотр и фотографирование гистологических образцов проводились с использованием микроскопа "Swift Instruments International", оборудованного камерой "Echoo-Imager 502000", с помощью компьютерной программы "TopView 3.2".

Общеморфологические исследования показали изменение формы и структуры ворсинок хоріона в исследуемых образцах, по сравнению с контрольными. На основе лектиногистохимических исследований выявили разное экспонирование рецепторов лектинов в структурных компонентах ворсинок хоріона 4-13-недельных эмбрионов человека. Самая высокая экспрессия рецепторов лектинов WGA, GNA и PFA обнаружена в исследуемой группе привычного невынашивания беременности, что указывает на нарушение конечных этапов гликозилирования биополимеров вследствие чрезмерной активации гликозидаз, по сравнению с группой спорадических выкидышей и контрольной группой.

Ключевые слова: ворсинки хоріона, спорадическое невынашивание, привычное невынашивание, лектиногистохимия, эмбрионы человека.

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МІКРОФЛОРА КИШЕЧНИКА ЩУРІВ ПРИ ЗАСТОСУВАННІ АВТОКЛАВОВАНИХ КАРОТИНОВІСНИХ ДРІЖДЖІВ ЗА УМОВ ЕКСПЕРИМЕНТАЛЬНОГО ДИСБАКТЕРІОЗУ

У статті представлено результати дослідження впливу автоклавованої біомаси каротиновісних дріжджів на стан мікробіоценозу кишечника щурів за умов експериментального дисбактеріозу, спричиненого введенням антибіотика. Показано, що при сумісному застосуванні автоклавованих дріжджів та доксицикліну нівелюється негативний вплив антибіотика на склад мікрофлори прямої кишки щурів та відбувається вирівнювання його до показників інтактних тварин.

Ключові слова: мікрофлора кишечника, автоклавовані каротиновісні дріжджі, експериментальний дисбактеріоз.

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Порушення екологічного стану навколишнього середовища проявляється і в негативних змінах у ендоекології кишечника людини та тварин. З відкриттям та широким застосуванням антибіотиків посилюється вивчення ролі мікрофлори кишечника у забезпеченні життєдіяльності макроорганізму. Так дослідження їх негативного впливу пов'язані, перш за все, із порушенням рівноваги у складі мікробіоценозу і виникненням передумов до розвитку інфекційних захворювань.

Для профілактики та лікування багатьох патологічних станів, що пов'язані із порушенням мікробіоти кишечника, широке застосування отримали пробіотики – препарати на основі живих клітин мікроорганізмів (лактобактерій, біфідобактерій, ентерококів, дріжджів) [4,5]. Основним завданням пробіотикотерапії було не знищення мікрофлори, а перерозподіл основних груп мікроорганізмів у екологічних нішах та забезпечення їх функціональної активності. Однак, потрапляючи у біотопи людини чи тварини, у жорсткі умови конкуренції із природною, добре адаптованою, мікрофлорою, комбінації штамів, вирощених у лабораторних умовах, гинули або значно знижували свою активність [8]. Тому у даний час, крім пробіотиків, для корекції мікробіоценозів шлунково-кишкового тракту застосовують пребіотичні речовини, що є селективними субстратами для окремих видів мікроорганізмів корисної мікрофлори кишечника. Найвідоміші з них фруктоолігосахариди, зокрема лактулоза [6]. Крім того, припускають, що при використанні пребіотиків відбувається посилення синтезу коротколанцюгових жирних кислот