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EMBRYO-CONDITIONED MEDIA ALTER THE EFFECTOR FUNCTIONS OF NATURAL KILLERS IN VITRO

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КОНДИЦИОНИРОВАННЫЕ ЭМБРИОНАМИ СРЕДЫ ИЗМЕНЯЮТ ЭФФЕКТОРНЫЕ ФУНКЦИИ ЕСТЕСТВЕННЫХ КИЛЛЕРОВ IN VITRO

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Abstract

NK cells represent the predominant lymphocyte population in the endometrium. NK cells engage in communication with the embryo during implantation, influencing implantation success. However, there is currently a lack of data in the literature regarding the influence of pre-implantation embryos on NK cell functional activity. The task of NK cells in the uteroplacental contact zone is to control trophoblast invasion, stimulate or limit it. Cytokines secreted by both embryo and NK cells —such as IL-1b, IL-6, IL-8, GM-CSF, and IP-10 — have been shown to stimulate migration and invasion of trophoblast cells Therefore, the purpose of this study was to develop a cellular diagnostic technology for predicting reproductive failure by investigating the effect of spent embryo culture media (ECM) on the cytotoxic activity of NK cells against trophoblast JEG-3 cells in an in vitro cytotoxicity model.

It was found that the death rate of Jeg-3 cells after co-culture with NK-92 cells was higher than the spontaneous death of Jeg-3 cells. Collectively, spent embryo culture media which were not divided into groups based on the quality of the embryos reduced the death of Jeg-3 cells when co-cultured with NK-92 cells. We have established that ECM from grade A (excellent- quality) embryos reduce the effector functions of natural killer cells against JEG-3 trophoblast cells. We assume that the embryos with the highest implantation potential (grade A) secrete factors that suppress NK cell cytotoxicity toward trophoblast cells, thereby promoting embryonic survival and successful implantation. Previously, we detected cytokines affecting NK cell functions IL-6, IL-8, IL-1b, IL-10, IP-10 and GM-CSF secreted by excellent quality embryos into a spent culture medium.

Thus, excellent-quality embryos with the greatest implantation potential secrete factors that modulate NK cell effector functions at the maternal–fetal interface. The method of assessing the quality of embryos using a functional model is generally consistent with the morphological system for assessing the quality of embryos and can be recommended for use in clinical practice.

Keywords: ECM, NK cells, cytokines, trophoblast, implantation, cytotoxicity.

Резюме

NK-клетки представляют собой преобладающую популяцию лимфоцитов в эндометрии. NK-клетки взаимодействуют с эмбрионом во время имплантации, влияя на успех имплантации. Однако в настоящее время в литературе недостаточно данных о влиянии предимплантационных эмбрионов на функциональную активность NK-клеток. Задача NK-клеток в зоне маточно-плацентарного контакта - контролировать инвазию трофобласта, стимулировать или ограничивать ее. Было показано, что цитокины, секретируемые как эмбрионами, так и NK—клетками, такие как IL-1b, IL-6, IL-8, GM-CSF и IP-10, стимулируют миграцию и инвазию клеток трофобласта. Поэтому целью данного исследования была разработка технологии клеточной диагностики для прогнозирования репродуктивных потерь на основе анализа влияния отработанной среды для культивирования эмбрионов (КЭС) на цитотоксическую активность NK-клеток в отношении клеток трофобласта JEG-3 в модели цитотоксичности in vitro.

Было обнаружено, что гибели клеток JEG-3 после совместного культивирования с клетками NK-92 был выше спонтанной гибели клеток линии JEG-3. В совокупности отработанные культуральные среды эмбрионов, которые не были разделены на группы в зависимости от качества эмбрионов, снижали гибель клеток линии JEG-3 при совместном культивировании с клетками NK-92. Мы установили, что КЭС эмбрионов класса А (отличного качества) снижают эффекторные функции естественных киллеров в отношении клеток трофобласта JEG-3. Мы предполагаем, что эмбрионы с самым высоким потенциалом к имплантации (класс А) выделяют факторы, которые подавляют цитотоксичность NK-клеток по отношению к клеткам трофобласта, тем самым способствуя выживанию эмбриона и успешной имплантации. Ранее мы выявили цитокины, влияющие на функции NK-клеток, IL-6, IL-8, IL-1b, IL-10, IP-10 и GM-CSF, секретируемые эмбрионами отличного качества в отработанную питательную среду.

Таким образом, эмбрионы отличного качества с наибольшим потенциалом имплантации выделяют факторы, которые модулируют эффекторные функции NK-клеток на границе системы мать–плод. Метод оценки качества эмбрионов с использованием функциональной модели в целом соответствует морфологической системе оценки качества эмбрионов и может быть рекомендован для использования в клинической практике.

Ключевые слова: КЭС, NK-клетки, цитокины, трофобласт, имплантация, цитотоксичность.

1 Introduction

Implantation plays a crucial role in the establishment of pregnancy [1]. Most reproductive failures are caused by impaired embryo development during the implantation stage [1]. Currently, morphological assessment of embryo quality is mainly used in clinical practice to evaluate implantation potential in IVF cycles. However, this approach has significant limitations and correlates poorly with actual embryo implantation potential [1, 2]. In this regard, one of the key priorities in reproductive medicine today is the development of an effective, non-invasive test that capable of individually predicting the chances of successful implantation for each embryo.

The interaction between the mother's immune system and the developing fetus represents a complex dialogue , in which the fetus demonstrates its antigens and the maternal body recognises and responds to them [3]. The maternal innate immune system serves as a key regulator of this process, establishing the unique immunological environment essential for successful pregnancy [3]. When an embryo implants into the uterus, inflammatory mediators are actively produced due to the involvement of lymphocytes. This leads to significant changes in the composition of endometrial cells preparing for the formation of the placenta [3].

CD56bright uterine natural killer (uNK) cells predominate in the endometrium. These cells constitute at least 30% of the total endometrial lymphocyte population, regardless of the phase of the menstrual cycle. Their quantity progressively increases during the secretory phase, and in early pregnancy, they can account for up to 70% [3].

During placenta formation, the chorionic villi attach to the basal layer of the uterine decidua, ensuring the establishment of adequate maternal blood supply. In the course of this process, trophoblast cells interact with a specialized subset of NK cells in the decidual membrane (dNK). dNK cells play a key in angiogenesis and remodelling of uterine spiral arteries, as well as in regulating the extent of trophoblast invasion into the uterine wall [3].

Thus, NK cells represent the predominant lymphocyte populationin the endometrium. NK cells engage in communication with the embryo during implantation, influencing implantation success.. However, there is currently a lack of data in the literature regarding the influence of pre-implantation embryos on NK cell functional activity. Therefore, the purpose of this study was to develop a cellular diagnostic technology for predicting reproductive failure by investigating the effect of spent embryo culture media (ECM) on the cytotoxic activity of NK cells against trophoblast JEG-3 cells in an in vitro cytotoxicity model.

2 Materials and methods

Embryosused in the study

ECM obtained from fragmented embryos (44±1 hour of cultivation) at the stage of 4 blastomeres were used as supernatants. The spent culture medium obtained from each embryo was aliquoted in a volume of 40 µl into two Eppendorf tubes in order to further analyze the effect of the supernatant on the cytotoxic activity

of NK-92 cells. The supernatants were frozen and stored at -80 0C. Spent culture media were collected from 22 embryos of 22 patients.

Cell lines

Cells of the NK-92 line (ATCC, USA) were used in the work. The cells were cultured in plastic vials for suspension cultures in accordance with the manufacturer's recommendations (ATCC, USA). During cultivation a complete culture medium was used with the addition of IL-2 500 IU of the following composition: aMEME sterile nutrient medium (Biolot, Russia) with L-glutamine with the addition of 10% inactivated fetal veal serum (ETS), 10% inactivated equine serum (DHS), 0.2 mM myoinositol, 0.02 mM folic acid, 2 mM L-glutamine, 50 mcg/ml gentamicin, 20 mM HEPES buffer, 0.1 mM mercaptoethanol (Sigma, USA). The cells of the NK-92 line were transplanted 3 times a week after 1-2 days.

Trophoblast cells of the JEG-3 line (ATCC, USA) were cultured in plastic vials for adhesive cultures in accordance with the manufacturer's recommendations (ATCC, USA). Cultivating JEG-3 cells were in complete culture medium of the following composition: DMEM sterile nutrient medium (Biolot, Russia) with the addition of 10% inactivated fetal veal serum (ETS), 100 U/ml penicillin and 100 mcg/ml streptomycin, 0.5 mM L-glutamine, 1% interchangeable acids, 1 mM sodium pyruvate (Sigma, USA). The JEG-3 suspension culture was replanted 2 times a week after 2-3 days. To disintegrate the monolayer of JEG-3 cells a mixture of trypsin (Biolot, Russia) and versene (Biolot, Russia) was used in a 1:1 ratio.

Cell culture and all experiments were carried out in a humid atmosphere at 37 °C and 5% CO2. The viability of cells during replanting and in experiments was assessed using a trypan blue solution, while it was at least 95%.

Evaluation of the cytotoxic activity of NK-92 cells against JEG-3 cells in the presence of ECM.

NK-92 (ATCC, USA) cell lines were used as effector cells and JEG-3 (ATCC, USA) cells were used as target cells. NK-92 cells were introduced in a volume of 25 µl and cultured in 96-well plates for suspension cultures. CES from quality A, AB, B and C embryos in a volume of 75 µl was added to part of the wells, reaching a concentration of 6*106 cells in 1 ml of culture medium. A medium for the cultivation of G-TL embryos in a volume of 75 µl was introduced into the control wells. 50 µl of JEG-3 cells prestained with carboxyfluorescein succinimidyl ether (CFSE) (Sigma, USA) were added to the wells with NK-92 cells, achieving an effector:target ratio of 10:1. The cells were then incubated for 4 hours in incubator. After incubation, the cells were washed and treated with propidium iodide (PI) solution (Sigma, USA). The effect of CES from 22 embryos of different quality was analyzed. The analysis was performed using a FACSCantoII flow cytofluorimeter (BD, USA).

Statistical processing of results

Statistical processing of the results was performed in GraphPad Prism 8.0.1. using the nonparametric Mann-Whitney U-test. Each dilution of embryo-conditioned media was analyzed in one repeat in each experiment (n=22). Each control sample was analyzed in three repetitions.

3 Results

It was found that the death rate of Jeg-3 cells after co-culture with NK-92 cells was higher than the spontaneous death of Jeg-3 cells (Figure 1). Collectively, CES which were not divided into groups based on the quality of the embryos reduced the death of Jeg-3 cells when co-cultured with NK-92 cells (Figure 1). When dividing CES into groups according to the quality of the embryos, it was found that CES from quality A embryos reduced the death of Jeg-3 cells (Figure 1). CES from quality AB, B, and C embryos did not alter the death of Jeg-3 cells after co-cultivation with NK-92 cells (Figure 1).

4 Discussion

We have established that ECM from grade A (excellent- quality) embryos reduce the effector functions of natural killer cells against JEG-3 trophoblast cells. This finding is consistent with existing literature. During physiological pregnancy, the cytotoxic activity of NK cells is downregulated due to an increased expression of inhibitory KIR receptors, NKG2A, ILT2/ITL4 [4, 5]. We assume that the embryos with the highest implantation potential (grade A) secrete factors that suppress NK cell cytotoxicity toward trophoblast cells, thereby promoting embryonic survival and successful implantation.

Previously, we detected cytokines IL-6, IL-8, IL-1b, IL-10, IP-10, and GM-CSF secreted by excellent quality embryos into a spent culture medium [6]. IL-8, IL-10, and IL-6 inhibit the cytotoxic activity of natural killers directly or indirectly by interfering with the action of stimulating cytokines. According to literature,IL-6 and IL-8 mediate the suppression of NK cell cytotoxicity in vitro, by promoting the dephosphorylation of STAT proteins via SHP-2 (Src homology 2 domain-containing protein tyrosine phosphatase-2) which can limit STAT5 activation and perforin expression [6, 7]. In addition, IL-6 and IL-8 reduce the surface expression of NKp30 and NKG2D activating receptors on NK cells, which leads to a decrease in their effector functions [6]. IL-10 is an important cytokine that regulates homeostasis and limits excessive NK cell activation. In this context, IL-10 may indirectly inhibit IFN-γ and TNF-α production by NK cells through suppression of IL-12, IL-15, and IL-18 secretion by antigen-presenting cells. [7].

The task of NK cells in the uteroplacental contact zone is to control trophoblast invasion, stimulate or limit it. Cytokines secreted by both embryo and NK cells —such as IL-1b, IL-6, IL-8, GM-CSF, and IP-10 — have been shown to stimulate migration and invasion of trophoblast cells [8, 9, 10].

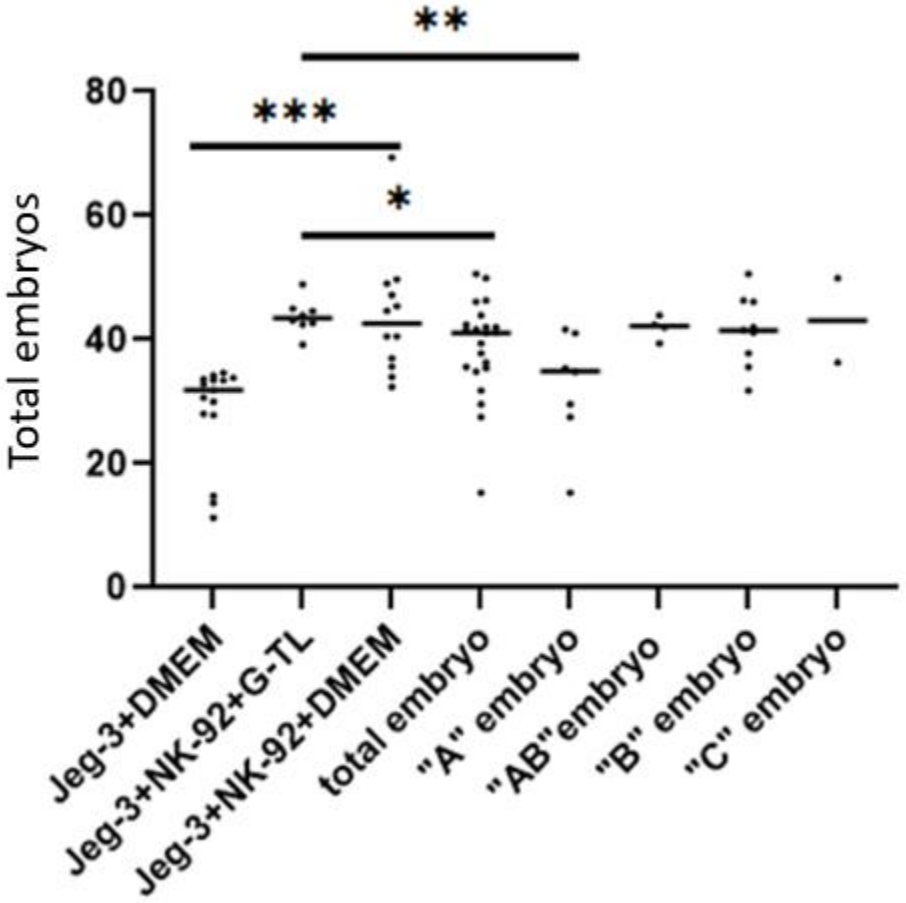
Previously, we found that CES from embryos of different quality have a different effect on the functional activity of endothelial cells, which is expressed in an increase in the intensity of proliferation and migration of endothelial cells involved in the formation of the vascular bed of the placenta [11].

Thus, excellent-quality embryos with the greatest implantation potential secrete factors that modulate NK cell effector functions at the maternal–fetal interface. The method of assessing the quality of embryos using a functional model is generally consistent with the morphological system for assessing the quality of embryos and can be recommended for use in clinical practice.

РИСУНКИ

Figure 1. Relative number of dead cells of the JEG-3 line in the presence of CES embryos of different quality.

Рисунок 1. Относительное количество погибших клеток линии JEG-3 в присутствии КЭС эмбрионов разного качества.



Jeg-3+DMEM (n=15) – spontaneous death of Jeg-3 cells cultured in DMEM medium; Jeg-3+NK-92+G-TL (n=8) - death of Jeg-3 cells cultured in DMEM medium and in G-TL in a 1:1 ratio medium in the presence of NK-92; total embryo (n=22) - death of Jeg-3 cells cultured in the presence of CES embryos of different quality without division into groups and DMEM in a 1:1 ratio when co-cultured with NK-92 cells; 'A' embryo (n=7) is the death of Jeg-3 cells cultured in the presence of quality A CES embryos and in DMEM medium in a 1:1 ratio when co-cultured with NK-92 cells; 'AB' embryo (n=4) is the death of Jeg-3 cells cultured in the presence of quality AB CES embryos and in DMEM medium in a ratio of 1:1 when co-cultured with NK-92 cells; 'B' embryo (n=9) - death of Jeg-3 cells cultured in the presence of quality B CES embryos and in DMEM medium in a ratio of 1:1 when co-cultured with cells of the NK-92 line; 'C' embryo (n=2) is the death of Jeg-3 cells cultured in the presence of quality C CES embryos and in DMEM medium in a 1:1 ratio when co-cultured with NK-92 cells. The significance of the differences: * - $p<0.05$; ** - $p<0.01$, *** - $p<0.001$.

Jeg-3+DMEM (n=15) – спонтанная гибель клеток линии Jeg-3, культивируемых в среде DMEM; Jeg-3+NK-92+G-TL (n=8) - гибель клеток линии Jeg-3, культивируемых в среде DMEM и в среде G-TL 1:1, в присутствии NK-92; total embryo (n=22) - гибель клеток линии Jeg-3, культивируемых в присутствии КЭС эмбрионов разного качества без деления на группы и DMEM в соотношении 1:1 при сокультивировании с клетками линии NK-92; ‘’A’’ embryo (n=7) - гибель клеток линии Jeg-3, культивируемых в присутствии КЭС эмбрионов качества А и в среде DMEM в соотношении 1:1 при сокультивировании с клетками линии NK-92; ‘’AB’’ embryo (n=4) - гибель клеток линии Jeg-3, культивируемых в присутствии КЭС эмбрионов качества АВ и в среде DMEM в соотношении 1:1 при сокультивировании с клетками линии NK-92; ‘’B’’ embryo (n=9) - гибель клеток линии Jeg-3, культивируемых в присутствии КЭС эмбрионов качества В и в среде DMEM в соотношении 1:1 при сокультивировании с клетками линии NK-92; ‘’C’’ embryo (n=2) - гибель клеток линии Jeg-3, культивируемых в присутствии КЭС эмбрионов качества С и в среде DMEM в соотношении 1:1 при сокультивировании с клетками линии NK-92. Достоверность различий: * - $p<0.05$; ** - $p<0.01$, *** - $p<0.001$.

ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

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Блок 3. Метаданные статьи

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КОНДИЦИОНИРОВАННЫЕ ЭМБРИОНАМИ СРЕДЫ ИЗМЕНЯЮТ ЭФФЕКТОРНЫЕ ФУНКЦИИ ЕСТЕСТВЕННЫХ КИЛЛЕРОВ IN VITRO

Сокращенное название статьи для верхнего колонтитула:

EMBRYO-CONDITIONED MEDIA ALTER THE FUNCTIONS NK CELLS
ЭМБРИОНАЛЬНЫЕ СРЕДЫ ИЗМЕНЯЮТ ФУНКЦИИ NK-КЛЕТОК

Keywords: ECM, NK cells, cytokines, trophoblast, implantation, cytotoxicity.
Ключевые слова: КЭС, NK-клетки, цитокины, трофобласт, имплантация, цитотоксичность.

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