SHORT MESSAGE

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EXPRESSION OF ESTROGEN AND PROGESTERONE RECEPTORS BY HUMAN ENDOMETRIAL MULTIPOTENT MESENCHYMAL STROMAL/STEM CELLS *in vitro* UNDER HYPOXIA CONDITIONS

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The aim of the study was to investigate the level of estrogen (ESR1, ESR2) and progesterone (PGR) receptors expression in the primary culture of endometrial multipotent mesenchymal stromal/stem cells endometrial multipotent mesenchymal stem cells and during *in vitro* cultivation under different athmospheric oxygen content. The dynamics of changes in the level of expression of the sex hormones receptors in the primary culture and during cultivation under different oxygen content in the athmosphere was shown.

Key words: human endometrium, multipotent mesenchymal stromal cells, expression of the receptors to the sex hormones, estrogen, progesterone.

Currently the problem of the reproductive function of the person violation is very relevant.

The endometrium plays a key role in the implantation process of the embryo. The uniqueness of the endometrium is not only in the ability to cyclic self-renewal of the cellular composition, but also in the ability to respond to changes in the hormonal status [1–3].

Functional and structural maturity of the endometrium is formed during the menstrual cycle in conditions of dynamic fluctuation of the level of steroid hormones of the ovaries — estrogens and progesterone [4] .The normal development of the endometrium and changes during the luteal phase are key to the successful implantation of the embryo [5, 6].

It is proved that determining the role in implantation is played by not only the concentration of steroid hormones acting on the tissue-targets of the reproductive system, but also the morphological structure and receptivity of the endometrium, that is, the number of functionally mature receptors for steroid hormones [7–11].

It is known that the concentration of oxygen in the tissues of the body and the niche of stem cells is much lower than atmospheric 20%. Hypoxia is one of the key factors that negatively affects the viability of transplanted cells and reduces their therapeutic potential. In addition, the concentration of oxygen in the atmosphere of the incubators during *in vitro* cultivation can affect the morphofunctional characteristics of cells in the culture.

The aim of the study was to investigate the level of estrogen (ESR1, ESR2) and progesterone (PGR) receptors expression in the primary culture of the endometrial multipotent mesenchymal stem cells (eMMSCs) and during *in vitro* cultivation under different atmospheric oxygen content.

eMMSCs obtaining and cultivating

Endometrial samples ($n = 5$) were obtained by biopsy in the proliferative phase of the menstrual cycle from women with endometrial hypoplasia. The age of patients was 34 ± 3.3 years. In all cases, the voluntary informed consent was signed. The fragments of the endometrium were dissociated by enzymatic treatment for 50 minutes in a solution of 0.1% collagenase IA and 0.1% pronase with the addition of 2% FBS (fetal bovine serum). The resulting suspension of cells was cultured in DMEM/F12 medium with addition of 10% FBS, 2 mM glutamine and 1 μg/ml of FGF-2 (all — Sigma, USA) in multi-gas incubators at 37 °C, absolute humidity, 5% CO_2 and 5% and 20% concentration of O_2. eMMSCs were selected as a cell fraction that adhered to the plastic after 24–48 hours after transferring the suspension to a culture vessel.

qRT-PCR

Total RNA was isolated from cells using TRIzol reagent (Sig ma-Aldrich, St. Louis, MO, USA) according to the manufacturer's protocol. Two μg isolated RNA were reverse transcribed to cDNA with RevertAid Reverse Transcriptase, RiboLock RNAase Inhibitor, and Oligo (dT) 18 anchored primer (all Thermo Scientific, USA). qRT-PCR was performed using Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific, USA) on a sequential detection system 7500 (Applied Biosystems, CA, USA) and analyzed by 7500 System SDS Software (version 1.3.1).The sequence of primers used in this study and PCR cycling conditions are listed in S2 Table. Expression of the TATA-box binding protein (TBP) was used as an endogenous control for standardization. Ct values were determined for the internal control (TBP) and the test genes at same threshold level in the PCR curves of the exponential phase. Relative quantification (comparative Ct (ddCt) method) was used to compare the expression level of the tested genes with the internal control and was represented in relative units. Dissociation curve analysis was performed after each run to check the specificity of the reaction. Three reactions (each in triplicate) were run for each gene, and the standard error of mean was calculated.

We have shown that cell populations derived from a minimal human endometrial biopsy were in line with the minimal criteria for the determination of MMSCs proposed by the International Society for Cellular Therapy [12]. The cells in culture were adhered to plastic under standard cultivation conditions and expressed at high levels of CD90+CD105+CD73+, in the absence of expression of hematopoietic markers CD34-CD45-HLA-DR-. Also, eMMSCs possessed the ability to direct trilinear differentiation in the adipo-, osteo- and chondrogenic directions, with the acquisition of the corresponding morphofunctional features [13].

The level of estrogen and progesterone receptors expression was determined in primary cultures, as well as in the course of cultivation over several passages (P0, P1, P3, P5) with 5% and 21% of O_2 content in the atmosphere.

The results of the ESR-1 expression level study are shown in Fig. 1. The level of estrogen-1 receptors expression (ESR1) during the cultivation was dynamically varied. In the primary culture of eMMSCs during P0, there was a significantly higher expression level of ESR1 under cultivation with 5% O_2 in the atmosphere, but this tendency was not maintained. An increase in the level of ESR1expression at oxygen content of 21% was observed already during P1. Subsequently, the level of expression was aligned and decreased in both groups of comparison.

The results of the ESR2 expression level study are presented in Fig. 2.

In the primary culture of eMMSCs during P0, the expression level of ESR2 was also higher in cultivation with 5% of O_2

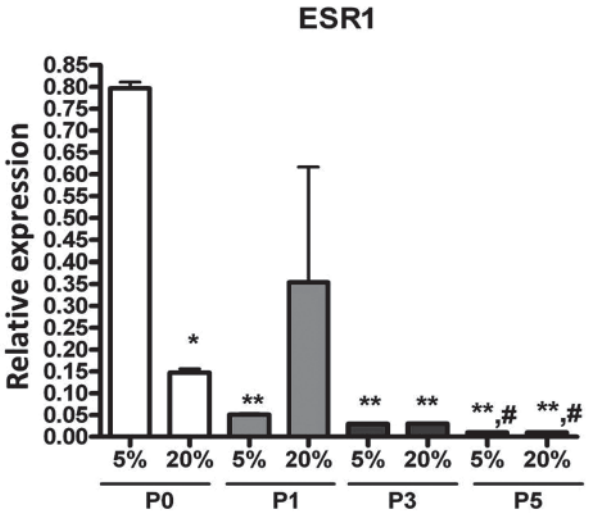

Fig. 1. **Expression of ESR1 estrogen receptor of eMMSCs in 20% and 5% O_2 cultivation with respect to the TBP household gene:**
* $P < 0.05$ in comparison with 5% of O_2 of corresponding passage;
** — $P < 0.05$ compared with 5% of O_2 during P0;
# — $P < 0.05$ compared with 5% and 20% of O_2, respectively, during P1, P3

in the atmosphere. The tendency towards equalization during cultivation was also maintained. It should be noted, that in contrast to the stable low expression of ESR1 in both groups during P5, an increase in the expression level of ESR2 in this passage was observed. It should also be noted that the cultivation of eMMSCs at 20% of O_2 during P5 results in a significant increase in the expression level of ESR2 compared to the primary culture and earlier passages.

Fig. 3 shows the results of the study of the expression level of eMMSCs progesterone (PGR) during cultivation.

The highest values of the level of the receptor to progesterone expression were noted in the primary culture of eMMSCs during cultivation with 5% of O_2 in the atmosphere. There was a tendency to decrease the expression of PGR in both groups during cultivation. There was no significant difference between the groups during P1, P3 and P5. However, the level of PGR expression in the primary culture during P0 was significantly higher when cultivated with 5% than 20% of oxygen in the atmosphere.

It should be noted that the level of estrogen and progesterone receptors expression differed in cultures depending on the donor.

Given the results of numerous clinical studies, the need to support the ART programs in the luteal phase with progesterone is beyond doubt. The glandular component of the endometrium is extremely sensitive to the action of progesterone and reacts in the first half of the luteal phase. The stromal component is less sensitive, reacts in the second half of the luteal phase and requires higher levels of progesterone.

It is known that estradiol and progesterone are the only hormones that are necessary to achieve the receptivity of the endometrium. Hormonal profile in DRT programs has features: too high levels of estradiol in the stimulation phase and before the prescription of a trigger dose of chorionic gonadotrophin, a sharp fall in estradiol levels after oocyte aspiration, lowered levels of progesterone in the luteal phase of the program. Due to clinical trials in different countries, it has been established that estradiol may not be added in the luteal phase in donation programs, but there is not enough data to suggest that this does not negatively affect the receptivity of the endometrium, the frequency of pregnancy and miscarriage.

Today, in clinical practice, the physiological levels of estradiol in the luteal phase are simulated and it is traditionally added to treatment regimens. Obviously, it is necessary to develop more precise criteria for the prescription of estradiol. One such criterion may be the ratio of the concentration of estradiol and progesterone, it is a better indicator than just the concentration of these hormones in blood plasma. However,

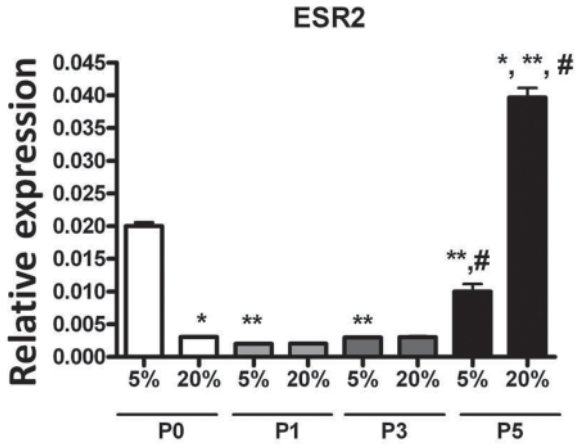

Fig. 2. **Expression of the ESR2 estrogen receptor of eMMSCs in 20% and 5% of O_2 cultivation with respect to the TBP household gene:**
* — $P < 0.05$ in comparison with 5% of O_2 of corresponding passage;
** — $P < 0.05$ compared with 5% of O_2 during P0;
# — $P < 0.05$ compared with 5% and 20% of O_2, respectively, during P1, P3

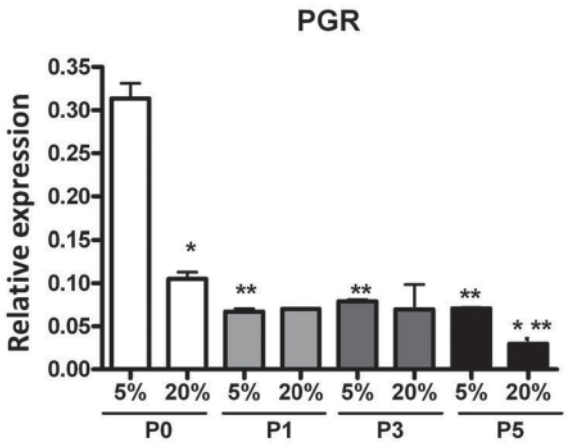

Fig. 3. **Expression of the PCR progesterone receptor of eMMSCs in 20% and 5% of O_2 cultivation with respect to the TBP household gene:**
* — $P < 0.05$ in comparison with 5% of O_2 of corresponding passage;
** — $P < 0.05$ compared with 5% of O_2 during P0

the concentration of hormones in the blood plasma does not provide exhaustive answers, because the presence of the hormone does not indicate its likely work, because for this purpose, the maturity of the target tissue and the willingness to respond to the stimulus are needed. That is why the study of the dynamics of vibration of receptors for hormones in eMMSCs *in vitro* is a promising field of study, as it can become the basis for optimizing the existing protocols for managing patients with unsuccessful ART attempts, which additionally applied cellular technologies to restore endometrial receptivity.

The work is experimental, therefore, it only represents the results of our research and does not outline the clinical picture in general, since it is the prerogative of gynecologists.

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ЕКСПРЕСІЯ РЕЦЕПТОРІВ ЕСТРОГЕНІВ ТА ПРОГЕСТЕРОНУ МУЛЬТИПОТЕНТНИМИ МЕЗЕНХІМАЛЬНИМИ СТРОМАЛЬНИМИ/СТОВБУРОВИМИ КЛІТИНАМИ ЕНДОМЕТРІЮ ЛЮДИНИ *in vitro* ЗА УМОВ ГІПОКСІЇ

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Метою роботи було дослідити рівень експресії рецепторів до естрогенів (ESR1, ESR2) та прогестерону (PGR) у первинній культурі ендометріальних мультипотентних мезенхімальних стромальних/стовбурових клітин і впродовж культивування *in vitro* за різного вмісту кисню в атмосфері. Показано динаміку зміни рівня експресії рецепторів до статевих гормонів у первинній культурі та протягом культивування за різного вмісту кисню в атмосфері.

Ключові слова: ендометрій людини, мультипотентні мезенхімальні стромальні клітини, експресія рецепторів до статевих гормонів, естроген, прогестерон.

ЭКСПРЕССИЯ РЕЦЕПТОРОВ ЭСТРОГЕНОВ И ПРОГЕСТЕРОНА МУЛЬТИПОТЕНТНЫМИ МЕЗЕНХИМАЛЬНЫМИ СТРОМАЛЬНЫМИ/СТВОЛОВЫМИ КЛЕТКАМИ ЭНДОМЕТРИЯ ЧЕЛОВЕКА *in vitro* В УСЛОВИЯХ ГИПОКСИИ

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Целью работы было исследовать уровень экспрессии рецепторов к эстрогенам (ESR1, ESR2) и прогестерона (PGR) в первичной культуре эндометриальных мультипотентных мезенхимальных стромальных/стволовых клеток и в течение культивирования *in vitro* в условиях разного содержания кислорода в атмосфере. Показана динамика изменения уровня экспрессии рецепторов к половым гормонам в первичной культуре и во время культивирования в условиях разного содержания кислорода в атмосфере.

Ключевые слова: эндометрий человека, мультипотентные мезенхимальные стромальные клетки, экспрессия рецепторов к половым гормонам, эстроген, прогестерон.