9. Shablii V.A., Kuchma M.D., Kyryk V.M. et al. Cryopreservation human placental tissue as source of hematopoietic and mesenchymal stem cells // Cell Transplantology and Tissue Engineering. – 2012. – Vol. VII, №1. – P. 54-62.

10. Terstappen L.W., Huang S., Safford M. et al. Sequential generation of hematopoietic colonies derived from single nonlineage-committed CD34/CD380 progenitor cells // Blood. – 1991. – Mar 15;77(6). – P. 1218-27.

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ПОРІВНЯЛЬНИЙ ФЕНОТИПІЧНИЙ АНАЛІЗ ПОПУЛЯЦІЙ ГЕМОПОЕТИЧНИХ ПРОГЕНІТОРНИХ КЛІТИН З РІЗНИМ РІВНЕМ ЕКСПРЕСІЇ *CD34*, ОТРИМАНИХ З ТКАНИНИ ПЛАЦЕНТИ І ПУПОВИННОЇ КРОВІ

Дослідження плацентарних гемопоетичних прогеніторних клітин (ГПК) і порівняння їх з властивостями ГПК плоду і дорослого організму необхідні для оцінки можливості їх клінічного застосування. Було показано, що тканина плаценти містить три популяції з різним рівнем експресії CD34, такі як $CD34^{+++}CD45^{low}$, $CD34^{++}CD45^{low}$ і $CD34^{+/low}CD45^{low}$. Як і в фетальній печінці, в плаценті містяться популяції з фенотипом $CD34^{++}CD45^{low/-}$ і $CD34^{+}CD45^{low/-}$, що дозволяє говорити про кровотворення в плацентарній тканині. $CD34^{++}CD45^{low/-}$ популяція також експресує CD133, практично негативна по лінійним маркерами і має лімфоцитоподібну морфологію. Це свідчить про те, що така популяція містить примітивні ГПК, потенційно – стовбурові клітини. Також серед плацентарних клітин присутні більш пізні прогенітори з фенотипом $CD34^{+/low}CD45^{+}$. Більшість таких клітин експресує гемопоетичні лінійні маркери. Популяція з фенотипом $CD34^{+++}CD45^{low}$ спостерігається лише в плаценті, клітини з таким фенотипом, імовірно, утворюються тільки в плацентарній тканині і/або мігрують з інших сайтів кровотворення, змінюючи при цьому рівень експресії CD34. Ферментативна обробка має незначний вплив на рівень експресії поверхневих білків при FACS аналізі, що варто враховувати в подібних експериментах.

Ключові слова: гемопоетичні прогеніторні клітини, плацентарний гемопоез, експресія CD34 маркера, пуповинна кров.

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СРАВНИТЕЛЬНЫЙ ФЕНОТИПИЧЕСКИЙ АНАЛИЗ ПОПУЛЯЦИЙ ГЕМОПОЭТИЧЕСКИХ ПРОГЕНИТОРНЫХ КЛЕТОК С РАЗЛИЧНЫМ УРОВНЕМ ЭКСПРЕССИИ *CD34*, ПОЛУЧЕННЫХ ИЗ ТКАНИ ПЛАЦЕНТЫ И ПУПОВИННОЙ КРОВИ

Исследование плацентарных гемопоэтических прогениторных клеток (ГПК) и сравнение их со свойствами ГПК плода и взрослого организма необходимы для оценки возможности их клинического применения. Было показано, что ткань плаценты содержит три популяции с различным уровнем экспрессии CD34, такие как $CD34^{+++}CD45^{low}$, $CD34^{++}CD45^{low}$ и $CD34^{+/low}CD45^{low}$. Как и в фетальной печени, в плаценте содержатся популяции с фенотипом $CD34^{++}CD45^{low/-}$ и $CD34^{+}CD45^{low/-}$, что позволяет говорить о кроветворении в плацентарной ткани. $CD34^{++}CD45^{low/-}$ популяция также экспрессирует CD133, практически отрицательна по линейным маркерам и имеет лимфоцито-подобную морфологию. Это свидетельствует о том, что такая популяция содержит примитивные ГПК, потенциально стволовые клетки. Также среди плацентарных клеток присутствуют более поздние прогениторы с фенотипом $CD34^{+/low}CD45^{+}$. Большинство таких клеток экспрессирует гемопоэтические линейные маркеры. Популяция с фенотипом $CD34^{+++}CD45^{low}$ наблюдается только в плаценте, клетки с таким фенотипом, предположительно, образуются только в плацентарной ткани и/или мигрируют из других сайтов кроветворения, изменяя при этом уровень экспрессии CD34. Ферментативная обработка оказывает незначительное влияние на уровень экспрессии поверхностных белков при FACS анализе, что стоит учитывать в подобных экспериментах.

Ключевые слова: гемопоэтические прогениторные клетки, плацентарный гемопоэз, экспрессия CD34 маркера, пуповинная кровь.

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DEVELOPMENT OF PIG CUMULUS-OOCYTE COMPLEXES AT CONSTANT AND OSCILLATING TEMPERATURE AND PH

It is shown that replacement of constant temperature and pH of culture conditions by oscillating ones does not significantly decrease diameter of COCs as a result of their maturation in in vitro culture for 24 hours. Increase in content of follicular fluid from 10 % to 20 % in the medium of their maturation NCSU does not influence the gain in COCs diameter.

Key words: cumulus-oocyte complex, follicular fluid.

Introduction. Improving the growth and development of the live object remains relevant because the problem associated with the increase of its productivity and improvement of its viability. You can select two opposite to each other, strategic approaches to its solution: genetic and epigenetic, which, like any opposites, deny and complement each other and are interconnected by passages. Gene expression takes place only in certain environments [23]. It is pointed out, in particular, by such concepts as expressivity and penetrance. The result of the development of a biological object is not only preformed, in particular, in the form of genetic program but also is determined by the epigenesis, in particular, by the influence of external conditions. And then, by the creation of conditions of the envi-

ronment, more adequate to the nature of the living object, one can (too) enhance its growth-development, contribute to the recovery of its (re)productive capacity. In this regard, previously unsolved, experimentally and theoretically, part of the overall problem of improving growth and development of a living object, the question remained, what should be a favorable external environment adequate, constant or inconstant, and what the character they should be if they are not constant.

In our view, between all known means of using the external environmental conditions for this purpose, the most adequate to nature of the living object, is the method of application, or at least maintaining namely biorhythmic oscillations of them. Namely it, as the literature data and our own research show, enables us to dramatically improve the growth-development and performance-viability of a living object, to remove it (re) productive potential.

Around the world, preimplantation embryos of mammals are continued to culture, in the hole, at constant conditions, which are trying to stabilize as strong as possible, – to prevent smallest changes, using a routine method which was developed 50 years ago [25].

It has been shown that the use of forced pH oscillations of culture medium, instead of it forced stabilization, significantly ($p < 0.001$) improves the development in vitro of 1 to 4-cell pig embryos ($n = 788$) flushed out from the organism: by 3.2 to 3.9 times more blastocysts are formed, and they grow to more advanced stages of embryonic development [4; 5]. Actually the survival rate of embryos increases.

As a result, the analysis of recent studies and publications, in which solving problems started, it was discovered that the theory and practice of application of the environment conditions oscillations, as opposed to the use of constant conditions, takes in the whole world more and more development and distribution.

It is shown that (biorhythmically) oscillating environmental conditions can be useful for improving the growth-development, performance-viability of the microorganisms, plants, crustaceans, amphibians [13], fish [9; 12], birds, [19; 20] and mammals [8].

For the present, oscillating environmental conditions have restricted use during in vitro culture of gametes, cells and mammalian preimplantation embryos [27; 28].

Here up to the present, they do not use oscillations of temperature and pH, indicating a sporadic and not a conscious application of oscillation on these biological objects, limited understanding of their role in the structures-functions of living object.

The aim of the work was to reveal the regularities of cumulus-oocyte complexes (COCs) growth in the maturation mediums in vitro – NCSU and 199 – with 10 and 20 % follicular fluid (FF) at biorhythmically oscillating temperature and pH in comparison with constant ones.

Materials and methods. The OCC were selected as an object of the study. Such a choice is conditioned by the need to increase the quantity and improve the quality of the oocytes in vitro maturation (in the form of COCs), from which embryos should further be get in vitro. The latest are used in large quantities for many scientific and practical purposes.

Of the dead at slaughterhouse pigs, the ovaries were withdraw and delivered to the laboratory, where the COCs and FF were received from the follicles.

Maturation of the COCs was done in the NCSU medium which were prepared with our own hands from Sigma reagents as described in literature, and in the medium 199, Sigma catalog number 5085 M.

10% (common) or 20% of the FF (in an attempt to make a step in the direction of the conditions that occur in vivo), 10 IU/ml of human chorionic gonadotropin, 10 IU/ml of horse chorionic gonadotropin, 0.53 mmol cysteamine and 20 mcg/ml gentamicin sulfate were added to the medium.

The in vitro culture at constant temperature and pH was carried out by a common (not principally modified for our purposes) method [25].

Cups with COCs in the appropriate medium, were put in gas chambers, – 100 ml of medical graded bottles with wide mouth, – blown through with gas mixture of carbon dioxide and air, which as a result of interaction with the medium has provided the last a constant pH around 7.4 units and tightly sealed.

The pH of the medium was controlled by measuring it in parallel chambers, which do not include the COCs. For this purpose, aliquots of the medium were taken and the pH of it was measured.

Temperature oscillation with 40-minute period was created by our own method [10; 17]. Air thermostat TC-80 was converted for this purpose into thermooscillator. Namely, the water in plastic bottles was placed into it and it was programmed to turn on and off using the timers of Feron and Brilux firms. The temperature changes in the chamber with COCs were judged from this in the parallel one, with a thermometer inserted into it, which pierces the wall of the thermooscillator chamber and shows of which can be watched at any time. Amplitude of temperature oscillation was changed from 37 °C to 39 °C by changing the amount of water in the thermooscillator. In another thermostat TC-80, temperature was maintained stable from 38.9 °C to 39.0 °C.

pH oscillation with daily period was created by our own method [11; 18]. To do this, specially constructed gas aluminum cameras with pipes of the silicone rubber half penetrated for gas were used.

COCs passed into the glass chambers with the maturation medium on which the vaseline oil was previously stratified. The initial pH of the medium, equal 8.0 to 8.2 unites, was created. These cameras put into gas chambers. The last were blown through with a mixture of carbon dioxide and the air, what led to a decrease in the medium pH up to 7.2. After 24 hours, the pH of the medium came up again the level of 8.0 to 8.2 unites owing to the back coming out of the carbon dioxide from the medium, and then doing so from the gas chamber, through the tube of silicone rubber, by the gradient of it concentration.

The stability of constant temperature in the range of 38.9 °C to 39.0 °C was guaranteed by thermostat TC-80. The COCs development was estimated by the change in magnitude of its diameter as a result of culture for one day. The diameter of the COCs was measured before and after culture using the eyepiece-micrometer of binocular microscope MBS-9. The relative growth rate, measured as a percentage, was counted by the formula Brody [15]:

$$y \equiv 2 \times 100 \times \frac{M_2 \div M_1}{M_2 \oplus M_1},$$

where y is the value of the y relative growth rate, expressed as a percentage, M1 is the diameter of the COCs before culture, M2 is the diameter of the COCs after 24 hours from the beginning the culture.

Biometrical processing was carried out on the computer using programs Excel and STATISTICA 6. All research was conducted in the Laboratory of Physiology of the Pig Breeding and Agro-Industrial Production Institute of NAAS of Ukraine.

Results and their discussion. 22 in vitro cultures were performed and 1249 COCs cultured. It is believed

that the sizes of living object related to the growth are distributed normally. In six of the ten studies, more than 100 COCs were generally employed.

This is the value which permits using parametrical statistics. On the other hand, it is known that nonparametric statistics have less statistical power (less sensitive) than their parametric competitors [16]. For these reasons, it was decided to be limited by the use of parametric statistics only.

As it can be seen from the results of this study, the percentage of the increase in the COC diameter in the NCSU medium with 10 % of FF at constant culture conditions does not differ significantly from that obtained as a result of culture in the same medium, as with 10 % of FF so with 20 % one, at oscillating temperature, separately – at oscillating pH, and received as a result of culture in the same medium with 10 % of FF at the oscillating temperature together with the oscillating pH (table 1).

***Table* 1. A comparison of the increase in the COCs diameter at constant and oscillating conditions in different maturation mediums contained different percentage of FF**

Culture conditions	Culture medium	Number of culture, n_1	Number of COCs, n_2	FF, %	Gain in COCs diameter, %	Cv, %
Constant	NCSU	13	185	10	48,28±3,98^{a}	28,52
	NCSU	5	75		50,92±6,97^{a}	27,36
	199	4	135		20,04±4,05^{c}	34,98
Oscillating temperature	NCSU	13	177	10	46,47±4,31^{ав}	32,06
	NCSU	5	77	20	48,67±7,21^{ав}	29,63
	199	4	139		33,50±4,59^{св}	23,70
Oscillating pH	NCSU	13	173	10	44,11±4,03^{ав}	31,63
	NCSU	5	80	20	42,57±4,15^{ав}	19,50
	199	1	46		32,93	-
Oscillating temperature an pH	NCSU	12	162	10	51,14±5,93^{a}	38,50

Note: the values with different superscripts differ significantly.

The percentage of increase in the COCs diameter in the medium 199 with 10 % of FF at constant culture conditions too differed not significantly from that obtained as a result of culture in the same medium with 20 % of FF at oscillating temperature. But the result obtained at oscillating temperature, had a distinct trend (although $p > 0.05$) to prevail that, resulted from constant conditions.

The percentage of increase in the COCs diameter in the medium NCSU with 10 % of FF at constant culture conditions was significantly greater ($p < 0.001$) from such derived as a result of culture in the medium 199 at the same culture conditions and with the same FF concentration. And at oscillating temperature, it differed not significantly.

The percentage of increase in the COCs diameter in the medium NCSU with 10 % of FF at oscillating temperature do not significantly differed from that obtained from culture in the same medium, but with 20 % of FF. The same applies to the culture at oscillating pH. Therefore, increasing the content of FF from 10 % to 20 % in the COCs maturation medium, NCSU, do not influenced negatively on increase of the diameter.

The data obtained are consistent with conclusion made [24], according to which the addition of FF to COCs maturation culture medium, prepared on the base of medium 199, can be beneficial, if is not more than 25%. So, in this way, one can save reagents in preparing medium for in vitro culture and the money at buying them.

It is interesting to compare the dimensions of the COCs and the magnitude of the diameter gain in our experiments with such in the works of other researchers. The average initial diameter of the COCs, which we took at the first thirteen cultures, was 15 points, or 210 microns. The average final diameter of the COCs was in the range from 25 units to 29 units, or from 350 mcm to 364 mcm. And the increase in the diameter of the COCs (Brody formulae) was in the range from 44 % to 51 %.

In experiments [22] the average diameter of the original COCs which were taken on culture in the medium that was also prepared on the medium NCSU, at the same concentration of FF (10 %), was equal to 240 microns, the average end – 340 microns.

In this case, the percentage increase in the diameter of the COCs in their investigation came up to the magnitude of 33 % which was less than that of received by us at least 11 %. However, they spent the measurement not after 24 h from the beginning of culture, but after 22 h.

But, they measured the largest diameter of the COCs, and we calculated the middle one. With this comparison, we can conclude that the culture conditions in our experiments were quite good.

The final diameter of COCs cultured by us (from 350 mm to 364 mm) coincides with this found [14]: diameter of the COCs, which had more than five – six layers of cumulus cells, composed from 210 microns to 350 microns. The following COCs are in the medium and large follicles.

At all culture conditions, a size of final diameter of the COCs strongly and almost everywhere, reliably correlated with the initial one (table 2). The regression coefficient also shows that practically all of the magnitude of the correlation is determined by the magnitude of the initial diameter of the COCs.

Low performance results of the COCs culture in the medium 199 at constant conditions can be explained by the fact that in this case it was less adequate to their needs than the medium NCSU, but the application of temperature oscillations (and, separately, also pH) significantly increased its adequacy.

The existence of differences in the expansion of cumulus depending on which medium is used for in vitro culture is known from the literature [21].

***Table* 2. The dependence of the final value of the average diameter of the COCs on the magnitude of their original middle diameter under different culture conditions**

# from the row-com	Culture conditions	Number of cultured COCs	The dependence of the final value of the average diameter of the COCs from the magnitude of the initial one		
			Correlation coefficient, r	Trustworthiness, p	Regression coefficient, r^2
1	constant, NCSU, 10 % of FF	185	0,82	0,0006	0,67
2	oscillating temperature, NCSU, 10 % of FF	177	0,94	0,0000	0,89
3	oscillating pH, NCSU, 10 % of FF	173	0,86	0,0002	0,74
4	oscillating temperature and pH, NCSU, 10 % of FF	162	0,88	0,0002	0,77
5	constant, medium 199, 10 % of FF	135	0,76	0,24	0,58
6	oscillating temperature, medium 199, 20 % of FF	139	0,92	0,085	0,84
7	constant, NCSU, 10 % of FF	75	0,98	0,0027	0,97
8	oscillating temperature, NCSU, 20 % of FF	77	0,93	0,0226	0,86
9	oscillating pH, NCSU, 20 % of FF	80	0,90	0,0386	0,81

It can be brined a few potential reasons of why the expected overwhelm in impact of oscillating parameters of culture medium is not detected in comparison with the influence of the constant ones.

One of them is a short duration of culture, for only the entire day. Usually, as the results of the literary review show, the positive impact of the factor is manifested after five or six days of the further culture. However, this culture duration was enough to show tendentious best influence of oscillating parameters of COCs culture medium, 199, compared with the influence of constant ones. The best effect of oscillating temperature on the increments of living mass, in comparison with the influence of constant one, was showed on young fish [9].

The second possible reason is no resonance (rhythm) changes in temperature in relative to the rate of growth and development of COCs (resonance rhythm is unknown).

The third one is the application of too large period of pH biorhythmic changes; it is desirable to experience the one hour rhythm of the pH oscillation in the future.

The fourth one is suboptimal amplitude of temperature and pH oscillations in some cultures of this searching research.

The fifth one is the use of medium designed for culture in namely constant conditions.

Comparison of the percent of the COCs diameter gain in medium NCSU and 199 led us to the realization of the fact that all, without exception (!), to the best of our knowledge, the existing culture medium for any cells and tissues, gametes and mammalian preimplantation embryos are designed with the aim of culturing them at stable constant conditions.

First of all we have in mind the medium 199 and the medium NCSU.

It may be one of the most important reasons of why it is difficult to show that the culture at oscillating conditions can be much more useful than the culture at constant ones.

Such utility should be displayed on medium, specifically constructed for this purpose, or on the medium that contains a significant amount of liquid of biological origin.

We think that the liquid of biological origin certainly needs to be such that reflects the biorhythmicity of the processes in the living body and its cells, and reflects the diversity of living objects at the cellular and subcellular level.

In our conception, in other to promote the culture at oscillating conditions, the medium would consist not only of those components, which are consumed by cells under conditions that occur near the position of conditional equilibrium around which medium conditions are oscillating on some of its parameter but also with those which are consumed by cells at the edges of the range of the environmental changes of the cells around the points of maximum deviations of these environmental conditions the cell from a conditional equilibrium, in extremes of their (conditions) sinusoidal changes.

Namely from these positions, it can easily be explained the tendentious preference applying oscillating parameters over constant ones only in the medium 199, which is much more complicated for the medium NCSU.

Why oscillation conditions of oocyte culture medium (also cells and embryos) can be more useful than constant ones?

In quantitative terms, oscillation of medium conditions is the transition from maximum concentration of a substance or maximum of medium parameter to minimum one or minimum of medium parameter, and vice versa, which is performed around conditional position of equilibrium, around which the oscillation takes place, around the average concentration, the average value of the parameter.

In qualitative terms, oscillation is the transition into relatively opposite state, for example: from acidic state to alkaline one and vice versa, if we talk about concentration of hydrogen ions, and hydroxyl ions; from warm to cold and vice versa, if we talk about the temperature.

It is well known that the rhythm is a universal feature of the movement of matter [1], each parameter of the body oscillates multiperiodically [6], oscillation is observed from early ontogeny: entrance of sperm cell into oocyte generates oscillation, in particular, calcium ions [29], and the external (environmental) conditions are oscillating and fluctuating too [3].

According to our vision, the creation of (biorhythmically) oscillating medium conditions, pH, temperature and other parameters, can synchronize processes in living object, to promote the maturation of eggs in time – alternate greater strengthening the growth and development, the anabolism and catabolism. After all, it is known that oscillations are necessary for cells to be passed from one extreme of the physiological state in which outweigh the anabolic processes in the second, where the katabolic ones is dominated [2].

(Biorhythmic) oscillatory mutual transitions of opposite states of medium (environmental) conditions (for example, temperature maximum and minimum or pH maximum and minimum) can contribute to the same (biorhythmic) oscillatory mutual transitions of the expression each of any opposites of any structure-function of the gametes (and also the cell, embryo, organism).

In contrast to constant conditions of environment, especially to those constancy of which stabilize at full strength, overly, oscillation extends the ranges of parameter changes, diversifies the environment on parameter values and, respectively, quality.

During the culture of 1 – 4-cell pig embryos in vitro constant pH was managed to hold within a range of ± 0.1 units, while oscillating pH changes in the range of 7.3 to 8.3 units [5].

It is well known that many different enzymes, as well as a variety of hormones, are presented in the cells of the organism by isomeric forms.

In the environmental conditions, (biorhythmically) oscillating in the normally width range (biologically diverse), unlike the constant ones, overly stabilized, narrowed, more different genes, whose products are formed isomeric forms, may be exposed to the expression.

In this case, in oscillating medium, the probability of one or other biochemical reactions processing may be increased.

If some gametes (or cells of an embryo or organism), have genetically altered some of the genes that are responsible for the formation of the isomeric form, enzyme can be at least is not active in constant environment (medium).

In the oscillating medium, same isomeric form can be formed, although only during some phases of the oscillatory period (cycle). And the reaction will be processed periodically.

In oscillatory conditions, a cell, perhaps, can live with the participation of a greater diversity of their genes, products of their expression, with a greater diversity of structures, built from these products, a greater variety of functions that are inherent to these structures.

In the oscillating (and fluctuating) medium conditions, biological diversity can be helpful on all structural and functional levels.

It can be assumed that, together with a temperature and pH oscillations, which we created in our work, the concentration of calcium ions was oscillated too what, as was shown by researchers, promotes gene expression in the cell [26].

As we expect, oscillatory culture, in comparison to culture at constant medium conditions, will give the possibility to develop normally in vitro to a larger percent of the total number of COCs taken on culture.

Still, replacing of constant culture medium parameters for oscillating expands conditions of environment and in this way allows to survive those biological objects, which need for their development environmental conditions that differ from those that are provided by the culture at constant, overly narrowed, stabilized conditions.

Not for all COCs optimal cultivation condition may be, for example, a pH of 7.4 units because all COCs are slightly different by nature. Has no doubt that discovered [7] biological non-equivalence and differential quality of reproductive cells, apply in the full measure to the COCs too.

The prospects for further development in this direction are large and multisided.

Still, in the case of the finding for optimal regimes of biorhythmic oscillation of medium culture conditions it can be used the new powerful nonspecific factors for in vitro development of oocytes and embryos, sperm capacitation, in vitro fertilization, inducement the oocytes in vitro to parthenogenetic development.

In this way is made, only the first, but very important steps: it is shown that the processes of COCs growth associated with getting embryos in vitro, can go under oscillating conditions at least at the same level as they do under constant ones.

The next step will be to find those ranges and amplitude of oscillation parameters of culture medium that will provide the best results with in vitro embryo obtaining from those that are received at constant conditions.

This step is already done for the preimplantation embryos received in vivo and cultured at pH, oscillated with the daily period [5].

In this work we leaned on biorhythms that are known for the somatic cells of the organism.

In further work, it would need to rely on the biorhythms that are place in oocytes during their maturing, but what we are not yet known.

Assume that the study on the culture at any (biorhythmically) oscillating medium parameters of any cells, taken from any plants and animals, will be perspective.

Such medium conditions could, in particular, to reduce the concentration of inoculate, to increase performance and viability of the cells.

An extremely promising is the direction of development the nutrient mediums for culture namely in oscillatory conditions.

Another promising line of research can be related with the development of equipment that can provide (biorhythmic) oscillation of temperature (thermooscillator instead of thermostat), pH (CO_2 incubator-oscillator instead of CO_2 incubator), lighting, electromagnetic field, concentration of gases in the atmosphere…

Conclusions: 1. The gain in COCs diameter, obtained after 24 h of culture in the medium NCSU at oscillating temperature and pH, both separately and together, does not trustworthily differ from the gain, received at constant conditions.

2. The gain in COCs diameter, obtained after 24 h of culture in the medium 199 at oscillating temperature has expressive trend (although $p > 0.05$) to be greater than the gain, received at constant conditions.

3. The gain in COCs diameter, obtained after 24 h of culture in the medium NCSU at constant conditions is significantly more than the gain, received as a result of culture in medium 199 at the same conditions.

4. Enhancement of the FF content from 10 % to 20 % in the maturation medium of the COCs, NCSU, does not influenced negatively on the increase of their diameter.

5. The advantage of oscillating culture conditions over constant ones expressively manifests itself in a relatively more complicated medium 199 compared to medium NCSU.

References

1. Алякринский Б.С., Степанова С.И. По закону ритма. – М., 1985.

2. Бюннинг Э. Биологические часы // Биологические часы. – М., 1964. – С. 11-26.

3. Григор'ев П.Є. Зв'язок інфрадіанної ритміки фізіологічних процесів у тварин з варіаціями геліогеофізичних факторів : автореф. дис. канд. біол. наук. – Сімферополь, 2005.

4. Денисюк П.В., Мартыненко Н.А. Принципиально новый метод культивирования доимплантационных эмбрионов млекопитающих // Доповіді Нац. АН України. – 1995. – № 11. – С. 148-149.

5. Денисюк П.В. Вплив pH середовища на розвиток in vitro доімплантаційних ембріонів свині : автореф. дис. … канд. біол. наук. – Харків, 1997.

6. Доскин В.А., Лаврентьева Н.А. Ритмы жизни. – М., 1991.

7. Квасницкий А.В. Половые клетки с.-х. животных, их биологическая неравноценность и разнокачественность // Науч. тр. Полтавский с.-х. ин-т. – 1949. – Вып. 4. – С. 79-92.

8. Квасницкий А.В., Мартыненко Н.А. Использование пульсирующей гипобарии для стимуляции развития эмбрионов у свиней и повышения их выживаемости // Физиол. журн. – 1980. – Т. 26, № 6. – С. 830-834.

9. Константинов А.С., Зданович В.В. Влияние осцилляции температуры на рост и физиологическое состояние молоди карпа (Cyprinus Carpio L.) // Доклады АН СССР. – 1985. – Т. 282, – № 3. – С. 760-764.

10. Корчан Н., Денисюк П. Розвиток ооцит-кумулюсних комплексів (ОКК) in vitro за осцилюючої температури // Вісн. проблем біол. і мед. – Полтава, 2011. – Вип. 1. – С. 250-252.

11. Корчан Н., Денисюк П. Розвиток ооцит-кумулюсних комплексів (ОКК) in vitro за осцилюючого pH // Вісн. проблем біол. і мед. – Полтава, 2012. – Вип. 1 (91). – С. 77-80.

12. Кузнецов В.А. Константинов А.С., Лукиянов С.В. Влияние колебаний pH на эмбрионально-личиночное развитие щуки Esox lucius // Усп. совр. биол. – 2009. – Т. 129, – № 3. – С. 286–293.

13. Лобачёв Е.А. Влияние колебаний экологических факторов на эмбрионально-личиночное развитие земноводных: дисс. канд. биол. наук. – Саранск, 2008.

14. Лобченко В.А. Влияние физиологических факторов на качество ооцитов свиньи и их созревание вне организма : автореф. дис. канд. біол. наук. – Харьков, 1991.

15. Brody S. Bioenergetics and growth. With special reference to the efficiency complex in domestic animals. – N. Y., 1945.

16. Непараметрическая статистика и подгонка распределения [Електронний ресурс]. – Режим доступу : http://www.statsoft.ru/home/textbook/modules/stnonpar.html.

17. Патент 62419, Україна, МПК (2011.01), A01N63/00. Спосіб культивування поза організмом ооцит-кумулюсних комплексів (ОКК) за температури, осцилюючої з одногодинним періодом / Корчан Н.О., Денисюк П.В.; заявник і патентоутримувач Інститут свинарства ім. О.В. Квасницького НААН; опубл. 25.08.2011, Бюл. № 16.

18. Патент 68013, Україна, МПК (2012.01), C12N 5/00, A61M 1/00. Застосування примусової біоритмічної осциляції pH середовища культивування поза організмом як способу, призначеного для збільшення міри розростання ооцит-кумулюсних комплексів (ОКК) / Корчан Н.О., Денисюк П.В.; заявник і патентоутримувач Інститут свинарства і агропромислового виробництва НААН. – № u 2011 10439; заявл. 29.08.2011; опубл. 12.03.2012, Бюл. № 5.

19. Патент RU № 2070387, Россия, A01K 41/00. Способ инкубации яиц сельскохозяйственной птицы / Фандеев Е.И. и др.; заявители и патентообладатели Фандеев Е.И. и др. – № 94028901/15; заявл. 03.08.1994; опубл. 20.12.1996.

20. Патент RU № 2107434, Россия, A01K 41/00. Способ инкубации яиц сельскохозяйственной птицы и устройство для его осуществления / Фандеев Е.И. и др.; заявители и патентообладатели Фандеев Е.И. и др. – № 94035822/13; заявл. 27.09.1994; опубл. 27.03.1998.

21. Abeydeera L.R. Development and viability of pig oocytes matured in protein free medium containing epidermal growth factor // Theriogenology. – 2000. – Vol. 54. – P. 787–797.

22. Beek J., Bijttebier J., Maes D. et al. The importance of cumulus expansion for monospermic fertilization of porcine cumulus-oocyte complexes // Reprod. Fertil. Dev. – 2011. – V. 23, N. 1. – P. 202.

23. Booth F.W., Chakravarthy M.V., Spangenburg E.E. Exercise and gene expression: physiological regulation of the human genome through physical activity // J. Physiol. (Lond). – 2002. – Vol. 543, Is. 2. – P. 399-411.

24. Brauch A., Keisler D.H. Effect of follicular fluid supplementation on in vitro maturation of porcine oocytes [Електронний ресурс] // Undergraduate Research and Creative Achievements Forum (MU) : collection. – 2007 [163]. – Режим доступу : http://hdl.handle.net/10355/1572.

25. Brinster R.L. A method for in vitro cultivation of mouse ova from two-cell to blastocyst // Exp. Cell Res. – 1963. – Vol. 32. – P. 205-208.

26. Dolmetsch R.E., Xu K., Lewis R.S.. Calcium oscillations increase the efficiency and specificity of gene expression // Nature. – 1998. – Vol. 392. – P. 933–936.

27. Mizobe Y., Yoshida M., Miyoshi K. Enhancement of cytoplasmic maturation of in vitro-matured pig oocytes by mechanical vibration // J. Reprod. Dev. – 2010. – Vol. 56, N. 2. – P. 285-290.

28. Ozil J. The parthenogenetic development of rabbit oocytes after repetitive pulsatile electrical stimulation // Development. – 1990. – Vol. 109. – P. 117-127.

29. Swann K., Lai F.A. A novel signaling mechanism for generating $Ca^2(+)$ oscillations at fertilization in mammals // BioEssays. – 1997. – V. 19. – P. 371-378.

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РОЗВИТОК ООЦИТ-КУМУЛЮСНИХ КОМПЛЕКСІВ СВИНІ ЗА ПОСТІЙНИХ І ОСЦИЛЮЮЧИХ ТЕМПЕРАТУРИ Й *РН*

Установлено, що заміна постійних температури й pH культивування на осцилюючі достовірно не зменшує приріст діаметра ооцит-кумулюсних комплексів (ОКК) у результаті їх дозрівання в культурі in vitro протягом доби. Підвищення вмісту фолікулярної рідини від 10 % до 20 % у середовищі їх дозрівання – NCSU, не впливає на величину приросту діаметра ОКК.

Ключові слова: ооцит-кумулюсних комплексів, фолікулярна рідина.

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РАЗВИТИЕ ООЦИТ-КУМУЛЮСНЫХ КОМПЛЕКСОВ СВИНЬИ ПРИ ПОСТОЯННЫХ И ОСЦЕЛИРУЮЩИХ ТЕМПЕРАТУРЫ И *РН*

Выявлено, что замена постоянных температуры и pH культивирования на осцилирующие достоверно не уменьшает прирост диаметра ооцит-кумулюсных комплексов (ОКК) в результате их дозревания в культуре in vitro на протяжение суток. Повышение содержания фоликулярной жидкости от 10% к 20% в среде их дозревания – NCSU, не влияет на величину прироста диаметра ОКК.

Ключевые слова: ооцит-кумулюсный комплекс, фолликулярная жидкость.

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EVIDENCE OF OXIDATIVE STRESS DEVELOPMENT IN PANCREATIC CELLS OF RATS WITH CHRONICALLY SUPPRESSED GASTRIC ACID SECRETION

Long-term hypochlorhydria is sometimes associated with mild pancreatitis development. Up to date, there is no clear evidence on mechanisms involved in pancreatic damage upon these conditions. The aim of study was to estimate the intensity of free-radical processes in rat pancreatic cells upon experimental hypochlorhydria. The increased hydrogen peroxide content (1,8 times), Nos2 gene mRNA level (2,9 times), total NO-synthase (4 times), thioredoxin reductase (1,6 times) and mitochondrial superoxide dismutase (1,5 times) activities, as well as decreased content of total (1,3 times), protein-bound (1,3 times) and nonprotein (1,4 times) SH-groups in rat pancreas were established. Thus, there is evidence of oxidative stress development in rat pancreatic cells upon long-term suppression of gastric acid secretion, suggesting the involvement of disturbed redox balance in pathophysiologic mechanisms of mild pancreatitis development upon these conditions.

Key words: oxidative stress, hypochlorhydria, dysbiosis, pancreas.

Introduction. Hypochlorhydria is defined as a state of low hydrochloric acid content in gastric juice with complex etiology: it develops as a consequence of pharmacologic suppression of gastric acid secretion, as a natural process during aging of digestive tract, as a complication upon some disease (atrophic gastritis, autoimmune disordrs, *H. Pylori* infection, achylia etc.) and in rare cases it has genetic causes [13].

In contrast to hyperacidic states, low stomach acidity is substantially harder to diagnose due to less prominent symptoms. At the same time, 10-15% of adult population and up to 50% people of retirement age in developed countries has hypochlorhydria [30]. Loss of gastric juice bactericidal properties is accompanied by bacterial overgrowth in different regions of gastrointestinal tract (GIT). Formation of dysbiotic bacterial biofilms in duodenum leads to initiation of endogenous inflammation that can involve associated organs, such as pancreas [3-4].

Although development of pancreatic disorders upon hypochlorhydria isn't unambiguous, there is some evidence of mild acute pancreatitis development (AP) upon long-term use of gastric H^+/K^+-ATPase inhibitors, such as omeprazole