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EXPERIMENTAL MODEL OF INDUCED LABOR IN CHRONIC PLACENTAL INSUFFICIENCY

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Summary: An experimental model for simultaneous registration of induced uterine contractile activity, electrocardiogram (ECG) of the female and fetuses developing intact in one horn and in conditions of reduced placental circulation in the other in chronic experience on female rabbits was developed. The model includes three stages. The first stage (on the 18th day of pregnancy) was to produce placental insufficiency by ligation of one third pre-placental vessels in one horn of the uterus, the other horn remained intact. It allowed us to compare reactions of normally developed and growth retarded fetuses. The second stage (28th day of pregnancy) was to register electromyography by electrodes insertion into the myometrium. Besides, for mechanohysterography an original sensor around the vaginal part of the uterus was placed and for electrocardiography electrodes were introduced into the muscles of interscapular region of normally developed and growth retarded fetuses. During the third stage (30th day of pregnancy) labor contractions of the uterus were induced by injecting of 1 IU oxytocin into an auricular vein of the female. Simultaneous registration of the uterine contractile activity and electrocardiograms of the female and its fetuses was performed. Approbation of the model was made on 16 female rabbits and 28 their fetuses, 14 of which developed in conditions of undisturbed placental circulation (intact fetuses) and 14 — in conditions of chronic placental insufficiency (experimental fetuses). It was shown that oxytocin-induced contractile activity of the uterus was different depending on a degree of the female biological readiness for delivery: in 8 females labor occurred and in 8 females did not. In the intact and growth retarded fetuses there were observed the fetal heart rate reactions, characteristic for those which develop in such fetuses during delivery in clinical situations. This model may be considered adequate for studying pathogenesis of the labor activity anomalies and intranatal hypoxia, as well as for preclinical approbation of the influence of various medicinal preparations on contractile activity of the uterus and on the functional state of the fetuses during labor.

Keywords: electromyography of the uterus; intrauterine pressure; female rabbit; pregnancy; placental insufficiency.

ЭКСПЕРИМЕНТАЛЬНАЯ МОДЕЛЬ ИНДУЦИРОВАННЫХ РОДОВ ПРИ ХРОНИЧЕСКОЙ ПЛАЦЕНТАРНОЙ НЕДОСТАТОЧНОСТИ

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Резюме: Разработана экспериментальная модель для одновременной регистрации в хроническом опыте индуцированной сократительной активности матки, электрокардиограмм (ЭКГ) беременной самки кролика и плодов, развивающихся интактными в одном роге и в условиях редуцированного плацентарного кровообращения в другом роге матки. Модель включает три этапа. На первом (на 18-й день беременности) создавали плацентарную недостаточность путем перевязки одной трети преплацентарных сосудов в одном роге матки, другой рог оставляли интактным. Такой подход позволял в дальнейшем сравнить реакции нормально развитых и отставших в развитии плодов. На втором этапе (28-й день беременности) проводили электромиографию с помощью введенных в миометрий электродов. Кроме того, оригинальный электрод располагали вокруг влагалищной части матки для механогистерографии и вводили электроды в межлопаточную область нормально развитого и отставшего в развитии плода для регистрации ЭКГ. На третьем этапе (30-й день беременности) путем инъекции 1 МЕ окситоцина в ушную вену самки проводили индукцию родовой деятельности. Осуществляли синхронную регистрацию сократительной активности матки и электрокардиограмм самки и ее плодов. Модель апробирована на 16 самках кролика и 28 плодах, половина из которых развивались в условиях ненарушенного плацентарного кровообращения (интактные плоды), а оставшиеся 14 — в условиях хронической плацентарной недостаточности (подопытные плоды). Показано, что индуцированная окситоцином сократительная активность матки зависела от степени биологической готовности самок к родам: у 8 самок произошли роды, а у 8 — нет. У нормально развитых и отставших в развитии плодов наблюдали реакции сердечного ритма, характерные для таких плодов в клинических условиях в родах. Описанную модель можно считать адекватной для изучения патогенеза аномалий родовой деятельности и интранатальной гипоксии, а также для доклинической апробации влияния разных медицинских препаратов на сократительную активность матки и функциональное состояние плодов в родах.

Ключевые слова: маточная электромиография; внутриматочное давление; самка кролика; беременность; плацентарная недостаточность.

INTRODUCTION

A large number of studies in modern obstetric literature are devoted to the antenatal fetal health protection. Questions of the fetal survival in the process of labor are covered to a lesser degree. It is well known that anomalies of labor activity remain the most typical obstetric complications of the delivery act, often causing the development of fetal hypoxia. Acute hypoxia occurs in 2–10.5% of all births and is one of the most common causes of adverse perinatal outcomes [1]. Thus, intranatal hypoxia is the main reason for surgical delivery in the fetal interests. In case of abnormal contractile activity of the uterus there appear intermittent episodes of acute fetal hypoxia, the response to which depends both on the frequency and intensity of the uterine contractions and on the functional state of the fetus. The most common syndrome in obstetric practice, that determines the degree of fetal reactivity disorder, including labor, is a chronic placental insufficiency, which is 30–77% of all pregnancies. Chronic placental insufficiency combines with the fetal development retardation and/or its growth restriction [17]. Fetuses with retarded development, compared with those normally developed, are more sensitive to hypoxia, what can lead to adverse perinatal outcomes: perinatal neurological diseases or even fetal death.

One of the ways to control abnormal uterine contractions and to normalize fetal functional state in the case of intranatal hypoxia is considered to be therapy with uterotonic, uterolytic, antioxidant / antihypoxic drugs. One of the obligatory stages of such study is a preclinical drug testing on laboratory animals. To date, however, there is no adequate model of chronic experience that allows simultaneous intrapartum registration of uterine contractile activity and functional state of normally developed and growth retarded fetuses.

Most experimental studies on the influence of placental dysfunction on the fetal growth and development were expensive and conducted on big laboratory animals such as monkeys, sheep and pigs. In some studies placental insufficiency was caused in mothers, in the others — in fetuses, and also there were models where it was caused both in mothers and fetuses simultaneously.

The objects of our study were rabbits, which have some advantages over other experimental animals. They are fast and easy to breed, available in determining gestational age. The rabbit placenta is of hemochorial type and therefore is close to human placenta because of its anatomic structure. The rabbit uterus is two-horned with separate innervation and blood supply what allows us to produce surgically placental blood flow insufficiency in one of the horns. Such intervention makes possible to observe reactions of retarded fetuses in one horn and intact fetuses in the other horn, compare them retrospectively with the same intensity of uterine contractions in the same maternal organism.

The goal of our research was to elaborate an experimental model of intrapartum hypoxia to allow simultaneous registration

of uterine contractions and electrocardiograms in fetuses which developed in conditions of normal placental circulation and in conditions of artificial placental insufficiency in chronic experience.

The most common cause of fetal hypoxia during labor is the umbilical cord compression and placental blood flow disturbance. Out of many models of placental insufficiency, described in the literature, we used a model which allows us to disturb the uteroplacental blood flow by means of dosed ligature of a part of preplacental vessels [22]. According to the author's data the dosed reduction of blood flow in preplacental uterine vascular branches by their partial ligature in one of the horns of the uterus leads to placental and fetal retardation as well as to impairment of their functional state in this horn on the 18th day of gestation.

When studying contractile activity of the uterus it is necessary to register simultaneously its electrical and mechanical activity. Some authors, in various types of animals, recorded electrical activity of the uterus, generated by muscle cells, using electrodes placed on the wall of the uterus or on the surface of the abdominal wall [2, 3, 5]. Electrical signal of the uterus positively correlates with intrauterine pressure [10, 15]. For registration of the intrauterine pressure in animal experiments there were used invasive methods: introduction of a catheter into the amniotic sac or of a small rubber balloon filled with saline solution into the uterine cavity [18, 19, 8, 9, 4]. These methods, combined with electromyography, gave the opportunity to investigate contractile activity of the uterus in chronic experiments on conscious animals without their fixing on the back. However, introduction of foreign bodies into the uterine cavity when registering its activity often leads to a disturbance of the course of pregnancy — premature delivery, burst of the amniotic fluid, uterine contractile activity disorder [16, 3, 10, 11].

It was necessary to devise a method of intrauterine pressure registration that would not influence the course of pregnancy and the state of the fetus while using synchronous recording of myometrial electrical activity as well as the fetal and female cardiac activity.

MATERIALS AND METHODS

Studies were performed on "Chinchilla" females (Oryctolagus cuniculus) with body weight 3000–4000 g. All animals were virgin and kept in regulated conditions on a standard food at the D.O. Ott Obstetrics and Gynecology Institute vivarium. Mating was carried out at the same time of a day. The next day after mating was considered as the first day of pregnancy.

The study was conducted in chronic experiments under standard conditions in accordance with the European Convention on the Protection of Vertebrates used for Experimental and Other Scientific Purposes (1990) and the ORDER of USSR Ministry of Health of August 12, 1977, № 755 "On measures to further improve the organizational form of the use of experimental animals".

The model creation consisted of several stages. At the first stage chronic placental insufficiency was produced. On the 18th day of pregnancy (the end of the placentation period) according to M.N. Vartanova's method aseptic ligature of 1/3 preplacental vessels was performed in one horn of the uterus under a mask narcosis (Diethyl ether, "Medhimprom", Russia) while the second horn remained intact. This period of rabbit pregnancy is approximately corresponding to the II trimester of human pregnancy. By this time organogenesis in rabbit fetuses is mainly completed, and then beginning from the 20th day an increase of the organs weight occurs [12]. Duration of the operation was about 15 min. After operation the animals were kept in vivarium in standard conditions.

At the second stage, on the 28th day of pregnancy, electrodes were introduced for simultaneous registration of contractile activity of the uterus and electrocardiograms of the female and fetuses being in one and the other horn of the uterus. The females were given intravenous thiopental narcosis (5% — 1.0 ml per 1.0 kg Thiopental sodium, "Synthesis", Russia). In aseptic conditions the female abdominal cavity was opened and vaginal part of the uterus was brought out into the operational wound. To eliminate the skin sensitivity, persisting in barbital anaesthesia, injection of 10 ml 1% novocaine solution (Procaine, "Organika", Russia) was preliminarily made into the abdominal skin in the intended region.

For the bipolar registration of electrical activity of the uterus two silver electrodes were inserted into the myometrium at a 0.5 cm distance from each other. The electrodes were connected with teflon-coated multithread cable and fastened to the uterus using ligature.

For registration of the intrauterine pressure we have used the sensor which was a thin elastic rubber catheter (2 mm in diameter and 2 cm long), filled with powered graphite as a conductor of an electric signal, both ends of which were connected with a multithread teflon line. This catheter in the form of a cuff was placed around the horn of the uterus at a distance of 1 cm from the electrodes, introduced for electromyography. Changes of the intrauterine pressure were judged about indirectly by the changing of the graphite resistance when the catheter strains during contractile activity of the uterus. The catheter was connected to the resistor bridge, which served to transform changes of electrical resistance of the sensor at the bridge entrance into the change of electrical resistance at its output.

To register fetal electrocardiogram a spear-shaped silver electrode (1.0 mm thick and 0.5 cm long) was introduced into interscapular region of the fetus through the intact wall of the uterus, fixed to its skin and to the uterine wall using ligature. An indifferent electrode, consisting of the silver plate, was placed on the muscles of the female abdominal wall. All electrodes were connected to labeled multiple-strand teflon cable which was stretched under the skin of the rabbit with the use of the probe leaded out between the ears. The cable was put into plastic box fixed to the skin. Duration of the operation was 10–15 minutes. After operation the animals were relocated into cages in a free position.

There were no cases of premature birth or death of the animals following surgical stages of the model creation.

The third stage was to induce the labor contractions of the uterus and its simultaneous registration with ECG of the female and its fetuses. Labor contractions of the uterus were induced on the 30th day of pregnancy by intravenous injection of 1 IU oxytocin

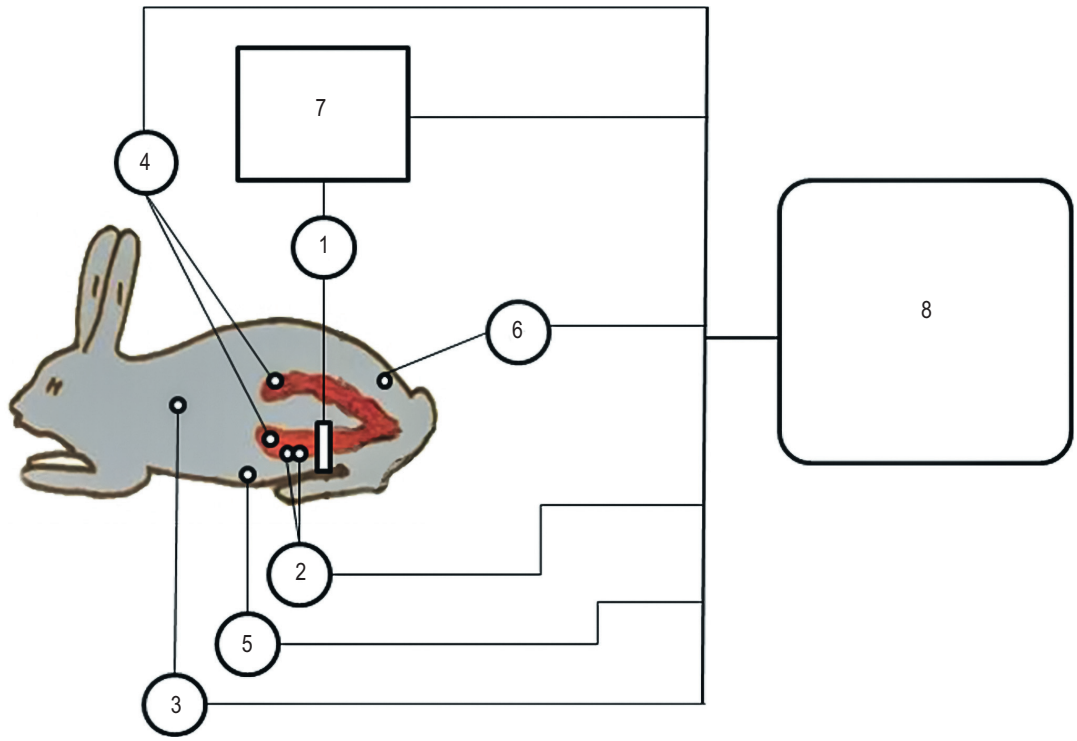

Fig. 1. **A scheme for simultaneous registration of electrical and mechanical activity of the uterus, female ECG and ECG of intact and experimental fetuses. 1 — original sensor with a sensitive element in the form of elastic cuff to register mechanical activity of the uterus; 2 — silver electrodes to register electromyograms; 3 — silver electrode for electrocardiograms in the female; 4 — silver electrodes for the fetal electrocardiograms; 5 — indifferent silver electrode in the form of a plate; 6 — reference needle-shaped steel electrode; 7 — resistor bridge; 8 — electroencephalographic computer complex "Diamant-EEG"**

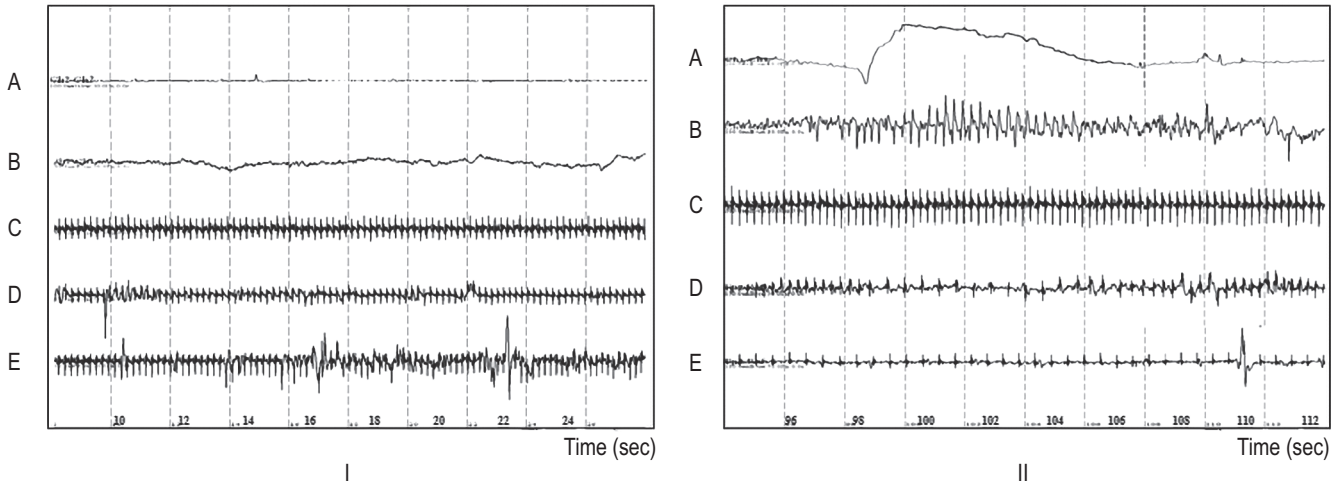

Fig. 2. **Registration on-line: I — basic value; II — 1 min after oxytocin injection (1 IU). A — intrauterine pressure; B — electromyogram of the uterus; C — electrocardiogram (ECG) of the female; D — ECG of growth retarded fetus; E — ECG of normally developed fetus**

("Gedeon Richter", Hungary). The choice of this term was defined by the fact that, as is known, in this species of animals till the term of labor the electrical activity of the uterus is either missing or is reduced to isolated potentials of different amplitude. Till the 29th day of pregnancy the uterus does not respond to oxytocin injections, even if it is given in large doses [13, 14, 6, 7].

During the experience the female was in a natural position in a special box, limiting its movements. Signals of electrical and mechanical activity of the uterus were registered simultaneously with synchronous recording of electrocardiograms in the female and fetuses with the use of electroencephalographic computer complex "Diamant-EEG" ("Diamant", Russia) and displayed in a real-time

regimen on computer with the help of special program adapted for our experiment. Wires from the electrodes and catheter were attached to a plant, the scheme of which is given in Fig. 1. The studied parameters were registered during 60 minutes.

As episodes of arising uterine contractions were considered those in which the amplitude of electric signal increased more than 3 times in relation to its basic value and which arose synchronously with the increase of intrauterine pressure. Figure 2 shows an example of recording electrical and mechanical activity of the uterus and ECG of the female and its fetuses before and after the introduction of oxytocin.

Analysis of the uterine activity was carried out for every 5-minute interval, it included the number of contractions, duration and amplitude of one contraction.

According to the ECG findings the mean fetal and maternal heart rate (HR) was calculated for each 10-second interval within one minute with an original computer program “Pulse” (Russia), based on the automated calculation of R-R-intervals of the ECG.

From experience the animals were removed after Thiopental anesthesia by means of aeroembolism. Localization of introduced electrodes was determined, fetuses and placentas were taken out and weighed.

The results were statistically processed according to parametric (Student's t-test) and non-parametric (Mann–Whitney U-test, Owen test, Wilcoxon test) statistics using standard package for statistical analysis (STADIA, STATGRAPHICS v.6). To determine the significance of results between groups Bonferroni multiple regression analysis was used.

MODEL APPROBATION

Testing of the model was carried out on 16 female rabbits and 28 their fetuses, 14 of which developed in conditions of normal placental circulation (intact fetuses) and 14 — in conditions of chronic placental insufficiency (experimental ones). In experimental fetuses weight was 20% less than that of intact fetuses (42.77±1.99 and 36.34±1.96 gr. respectively, $p < 0.05$).

In two groups weights of placentas did not differ.

Induction of the uterine activity with 1 ED of oxytocin on the 30th day of pregnancy resulted in labor in 8 females, in other 8 animals contractile activity of the uterus gradually decreased, and there was no labor in them. The model shows that induced uterine activity, judging by the number of contractions and duration of one uterine contraction in the females in which deliveries occurred, was significantly higher than in those with no labor. According to regression analysis, the number of contractions and the rate of their changes in the females in labor were significantly higher than in the females in which labor did not occur (Fig. 3). Various reactions of females on the introduction of oxytocin are connected, obviously, with different biological readiness for delivery due to unequal sensitivity of myometrial receptors to oxytocin. A change of the fetal heart rate after oxytocin injection was an indication of the fetal functional state.

After oxytocin injection (1 IU) the number of uterine contractions and the rate of their changes was higher in the females with induced labor than in the females without uterine activity ($p_1 < 0.001$, $p_2 < 0.001$, respectively).

It was shown in our model that the response to oxytocin injection and to the onset of uterine labor contractions of the intact and growth retarded fetuses in the females, who delivered, and those that did not was unidirectional and comparable: they have developed bradycardia. In the females who delivered fetal heart rate decreased in the intact and growth retarded fetuses by 46% and 41%, respectively, and in females that did not deliver in both groups of fetuses — by 40%. The observed heart rate response to oxytocin in the intact and experimental fetuses is corresponding to that observed in the development of the fetal intranatal hypoxia in clinical practice. Later on we considered as the main marker of the reactivity of fetuses the recover of their heart rate to the basic value.

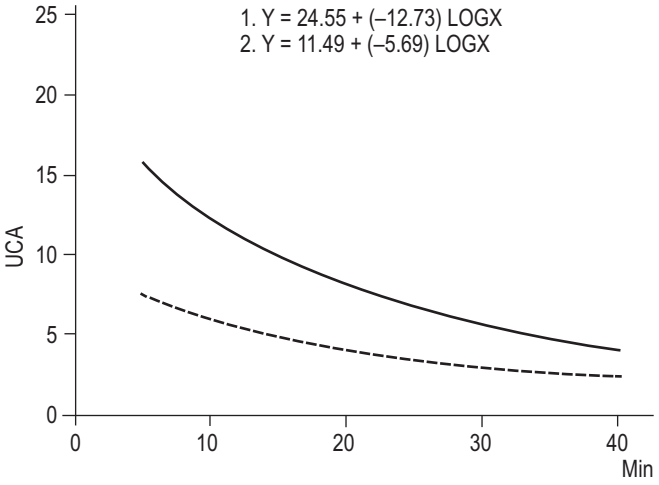

Fig. 3. The number of uterine contractions and the speed of their changes in females that were (solid line) and those that were not (dash line) in induced labor

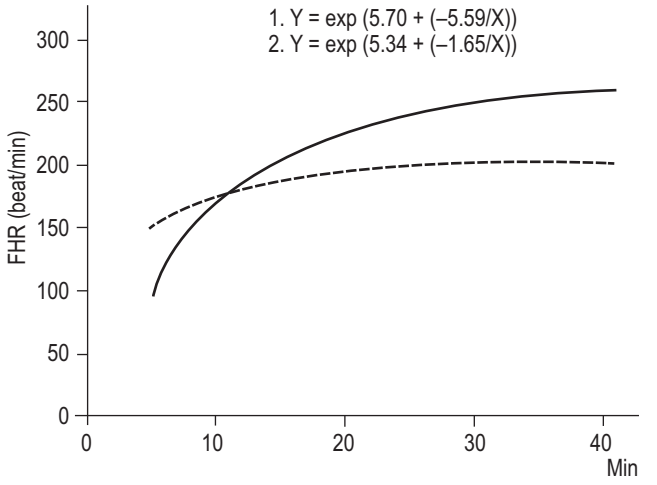

Fig. 4. Fetal heart rate and its dynamics in the intact (solid line) and growth retarded (dash line) fetuses of the females in induced labor

It was shown that the extent of the heart rate changes in the intact and experimental fetuses directly depended on the intensity of the uterine contractions. In case of active uterine contractions resulting in labor, bradycardia in growth retarded fetuses in response to oxytocin remained till the end of observation, in the intact fetuses their heart rate gradually increased and reached its basic value by the end of the study. According to a regression analysis, in the intact fetuses in comparison with the growth retarded ones the heart rate value and the rate of its dynamics change were significantly higher ($p_1 < 0.0009$; $p_2 < 0.01$, respectively) (Fig. 4). In the females without biological readiness for labor on the first stage the reaction of all fetuses was identical: their heart rate recovered to the basic level. Then in the intact fetuses tachysystole developed, while in experimental fetuses their heart rate remained at the basic level. However, regression analysis showed that the heart rate value in intact fetuses during observation was significantly higher, than in experimental ones ($p = 0.01$).

The heart rate and its dynamics in intact fetuses was higher than in growth retarded fetuses ($p < 0.001$, $p < 0.01$).

Our data are consistent with the literature data, obtained in clinical studies, where it was shown that during uterine contractions the heart rate of normally developed fetuses recovered faster in comparison with that in growth retarded fetuses [20]. In this situation, every next contraction of the uterus may result in blood acidosis followed by irreversible changes in the cardiovascular and central nervous system in the growth retarded fetuses [21].

CONCLUSION

Results of the presented model approbation have shown that the oxytocin induced contractile activity of the uterus depended on a degree of biological readiness of the female for labor. In the rabbit females with different biological readiness for labor, the heart rate reactions of the intact and growth retarded fetuses were comparable with those in such fetuses in clinical situations during labor. So, this model is possible to be considered adequate for studying pathogenesis of the labor activity and intranatal hypoxia, as well as for preclinical approbation of the influence of medicinal preparations on contractile activity of the uterus and on the functional state of fetuses.

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