THE CHANGES OF PLACENTAL ULTRASTRUCTURE IN PREGNANT RATS WITH SONOVUE CONTRAST AGENT

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ABSTRACT

Objective: *At present, the application of contrast-enhanced ultrasound (CEUS) in the diagnosis and research of gynaecological diseases has provided very beneficial effects. However, the clinical safety of the contrast doses administered during CEUS needs to be studied further. Therefore, this study aims to observe changes in the placental ultrastructure in pregnant rats injected with SonoVueTM contrast agent.*

Methods: *Thirty-six pregnant rats were randomly divided into three groups: a blank control group, a high-dose group and a low-dose group. A colour Doppler ultrasound diagnostic instrument and CPS were used for low mechanical index contrast and SonoVueTM was used as the contrast agent. The whole perfusion process was observed and recorded, and a time-intensity curve for the low-dose group was constructed. Changes in the placental barrier and the ultrastructure of the tissue cells were observed under a transmission electron microscope. Statistical differences between the groups were calculated using the SPSS Statistics 20.0 software package.*

Results: *After 15-26 seconds, the blood vessels on the maternal surface of the placenta were evenly diffused, which was the maximum value of video intensity. Half a day after the SonoVueTM injection, there were no obvious pathological changes in the placental ultrastructure between the blank group and the low-dose group.*

Conclusion: *The electron microscope showed that only the bottom layer of placental tissue in the high-dose group was slightly thicker, indicating that a low dose of SonoVueTM is safe for foetal disc imaging in late-term pregnant rats.*

Keywords: *SonoVue™ contrast agent, contrast-enhanced ultrasound, late pregnant rat placenta, ultrastructural changes.*

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Introduction

Although there have been many recent studies regarding the safety of contrast-enhanced ultrasound (CEUS) in the placenta of pregnant mice, it is not clear whether SonoVueTM can enter the foetal internal circulation through the placental barrier or whether it will adversely affect the foetus itself. Therefore, its application in obstetrics is currently restricted. At present, only patients with terminal foetal malformations, placental lesions or an otherwise unviable foetus, or who are suffering from postpartum placenta accreta need to terminate can have CEUS. CEUS can fully show the perfusion characteristics of uterine serosa, the myometrium, and healthy and diseased placentas both before and after delivery. It can also locate the placental position, can determine the relationship between the placenta and the muscular layer, distinguish the residual placenta from blood in the lumen, and overcome the shortcomings of conventional ultrasound in a placenta accreta examination. CEUS shows that the boundary between the placenta and myometrium disappears, and the myometrium becomes thinner during placenta implantation. It provides important information for clinical diagnosis and is conducive to selecting the correct treatment methods.

Ferraioli reviewed the application of CEUS and SonoVueTM (Bracco imaging) in the diagnosis of focal liver lesions (FLLS), the guidance of ablation treatment and the follow-up of liver tumours. Ferraioli used CEUS combined with traditional grey-scale ultrasound to analyse the percutaneous treatment of malignant liver tumours. However, although this method is comprehensive, errors can be made(1). In order to investigate the effectiveness of different sensors in the simultaneous interpreting of breast CEUS using SonoVueTM as a contrast agent, Wang performed a study on 51 patients with breast lesions using both a CEUS probe and a C5-1 probe. Wang's study used CEUS to investigate the effectiveness of the simultaneous interpreting of different sensors in breast CEUS using SonoVueTM as a contrast agent. The experimental requirement of the method is very high(2). Yousef divided 60 full-term placentas into three groups: a control group (Group I) consisting of normal placentas; an uncontrolled diabetes mellitus (Group II) group, where the placentas were from women with poor glycaemic control during pregnancy; and a controlled diabetes group (Group III), which included placentas of diabetic women whose blood sugar was controlled during pregnancy. Yousef's study used the group control method to explore the influence of diabetes on the development and function of the placenta – a method that is highly feasible but lacks stability(3).

Gvetadze conducted a study on 12 patients with t1-2cn0 oral tongue cancer using SonoVueTM contrast agent to record the location and imaging features of sentinel lymph nodes. Their study explored the application value of CEUS combined with peritumoral injections of microbubble contrast agent. This method has high stability but requires a high level of accuracy(4). In this study, 36 pregnant rats were randomly divided into three groups: a blank control group, a high-dose contrast medium group and a low-dose contrast medium group, and the entire perfusion process was observed and recorded. In addition, a time-intensity curve for the rats in the low-dose group was constructed. The ultrastructural changes of placental barrier and tissue cells were observed under a transmission electron microscope (TEM). Half a day after contrast injection, no obvious pathological changes were observed in the placental ultrastructure of the control group and the low-dose group. Under the TEM, the placental ultrastructure of the high-dose group was only slightly thickened, indicating that a low dose of SonoVueTM is safe for placental imaging in late-term pregnant rats.

Theory of unsaturated fatty acids and exercise

Sonovue and contrast-enhanced ultrasound

Synopsis of SonoVue™

Contrast-enhanced ultrasound (CEUS) is an advanced research area in ultrasound medicine and is considered to be a functional imaging technology. SonoVue™, which is a new generation of contrast agent, is a pure-blood pool tracer with a microbubble radius of only 1.25 μ M. It can visualise tissue microvessels and display the mass to the microvascular level. In the process of a routine ultrasound examination, SonoVue™ was injected into a peripheral vein to enhance the blood flow signal of the body and to enhance the sensitivity of slow bleeding and small blood vessels in human tissue(5). Using contrast can obtain more diverse and accurate data than colour Doppler ultrasound. SonoVue™'s placental CEUS is still in the initial stage, although its modal and quantitative analysis parameters can allow a clinician to more accurately evaluate benign and malignant lesions.

SonoVue™ is a contrast agent that cannot flow through the placental barrier and remains entirely within the maternal circulatory system. In theory, when this kind of microbubble cavitation exists in the villi space for a short time, the resulting high temperatures and pressure will cause the maternal blood vessels to contract, reducing the villi space and causing the syncytiotrophoblast nucleus near the villi to be broken. This can lead to nucleolysis and other forms of cell damage, and the vascular cavity in the villi will become congested(6). However, the pathological changes mentioned above were not observed in the placental microstructure of the rats in the high-dose and low-dose contrast medium groups, which is consistent with the practical results of using CEUS to observe microscopic pathological changes in other parts of the body.

Contrast-enhanced ultrasound (CEUS)

CEUS is the process of using an intravenous ultrasound contrast agent during a conventional ultrasound examination to improve human blood flow signal, constantly observe microvascular perfusions, improve the detection rate of lesions, distinguish between benign and malignant lesions, and enhance the specificity and sensitivity of ultrasound diagnosis(7). CEUS is a new non-invasive, non-ionising radiation imaging technology that can improve the sensitivity of slow blood flow in human tissues and display the microvessels more prominently. It can be used in the overall evaluation of various diseases. In addition, there are many advantages in evaluating the therapeutic effect of interventional therapy. Because there is no ionising radiation, the scans can be checked in real-time and can be repeated as many times as is necessary.

This is conducive to improving the effects of treatment and relieving pain in patients. CEUS microvascular imaging technology can track the whole perfusion process of microbubbles in tumour tissues and microvessels in real-time and can detect the blood flow perfusion, microvascular morphology, walking and overall distribution of human tissues, showing the time series and spatial distribution of microvascular perfusion and their corresponding differences, and reflecting the perfusion of human tissues at the microcirculation level(8). SonoVue™ contrast agent can be used to identify postoperative recurrence and traces of disease, assist in guided needle aspiration biopsies and assist in evaluating the efficacy of non-surgical treatment of diseases.

The placenta

Overview

The placenta is the key organ in maintaining the growth and development of the foetus in the uterus. Some of its most important functions include gas exchange, nutrient supply, metabolic waste excretion, immune defence and synthesis of various hormones. The placenta is a temporary organ during pregnancy. It is the place where the foetus receives oxygen and nutrients from the mother. Organelles are the functional units of cells, which contain the enzymes required for electron transport, nutrient synthesis and catabolism, and are also the key sites for oxidative phosphorylation and metabolism in cells(9). Therefore, its structure and function are very important for maintaining the healthy physiological function of cells, particularly for the syncytiotrophoblasts found in the placental cells.

Influence of placental morphology on pregnancy

The placenta is the intermediary place of maternal and infant nutrition and information exchange. Abnormal placental morphology often leads to a weakening in the placental functions, and eventually leads to a series of negative effects. Placenta and accessory placenta are common manifestations of abnormal placental morphology. Many practical conclusions have shown that any pregnancy is prone to perinatal complications such as placental abruption, premature delivery, lack of amniotic fluid and intrauterine foetal death(10).

A possible reason for this is that there are some hypoplastic blood sinuses on the edge of the placenta, which are connected with the chorionic space, meaning that it is not easy to pinpoint the source of bleeding during a placental rupture, which can result in incomplete placental function and reduced foetal renal perfusion. Similarly, the paraplacenta can cause postpartum haemorrhaging, significantly increasing the incidence of foetal death, which is related to the characteristics of the placenta previa and intrauterine residue(11). In addition, and different from the perinatal results of a single pregnancy, a twin pregnancy with abnormal placental morphology will not lead to placental abruption, vascular previa, placenta residue or other adverse pregnancy outcomes, which may be related to the small sample, twin pregnancy.

The caesarean section rate in foetal pregnancies is high, which indicates that the placental volume is positively proportional to an adverse pregnancy outcome. When the placental volume is the same, a wide, flat placenta is more likely to cause pet, premature delivery, high-risk infants and other adverse outcomes.

Ultrastructure

In the normal group, there were more microvilli on the free surface of the syncytiotrophoblasts. The mitochondria in the cytoplasm are round or rod-shaped, while the internal plate-shaped ridge is clear and thick(12). In the complex endoplasmic reticulum, the nuclear surface is large and irregular, the chromatin is closely connected and concave, and there are more subnuclear membranes and fewer zygotic nodules. A decrease in the number of placental villi is not conducive to material exchange between mother and infant. Mitochondria are the key energy supply stations of cells. If the mitochondria are damaged or reduced, or even lead to cells unable to synthesise, ATP induced plasma membrane ion pump dysfunction, which is required to maintain general efficiency, will cause water to enter the cytoplasm, resulting in cell oedema or necrosis.

Methods

Experimental design

Subjects and groups

This study used 36 healthy Wistar rats in the third trimester of pregnancy, with a gestational period of 15-22 days and body weights of 245-315 g. They were randomly divided into three groups:

• Blank control group: Saline (2.5 ml/kg) was injected into the caudal vein.

• High dose contrast medium group: 23.5 mg/kg SonoVueTM (2.5 ml/kg, 11.6 mg/ml) was injected.

• Low dose contrast medium group: 0.235 mg/kg SonoVueTM (2.5 ml/kg, 0.116 mg/ml) was injected.

Instruments and methods

Colour Doppler ultrasound with contrast pulse sequences (CPS) was used to evaluate the CEUS technology. When using the CPS technique, the probe frequency was 7.5 MHz, the mechanical index was 0.22, and the maximum dynamic range was 82 db. SonoVueTM was used as a contrast agent. The experiment proceeded as follows.

The pregnant rats were secured to the operating table in the decubitus position according to the groups. The back skin was anaesthetised, and a small amount of coupling agent was applied to the lower abdomen. The contrast medium (or normal saline) was injected according to the dosage. The enhancement and regression of the placentas of the 36 pregnant rats were observed for approximately six minutes. The entire contrast perfusion process was recorded, and a time-intensity curve for the low-dose group was constructed.

Thirty-six pregnant rats (12 in each group) were randomly selected to take placental tissue immediately after angiography. The placental tissue near the foetal side of each pregnant rat was fixed, embedded and sliced before being observed under a TEM to determine whether there were any changes in the placental barrier and ultrastructure of the tissue cells.

Statistical analysis

The calculation results were expressed as mean standard deviation using the SPSS Statistics 20.0 software package. The difference between groups was analysed by paired sample t-tests, and the significance level was taken as 0.05. The formulae used are shown below.

• Mean difference:

$$A.D. = \frac{\sum |x - \bar{X}| f}{\sum f} \quad (1)$$

• Standard deviation:

$$\sigma = \frac{\sum (x - \bar{X})^2 f}{\sum f} \quad (2)$$

Experimental analysis

Comparison of heart rates of the rats in each group ($\bar{x}\pm s$)

In order to accurately evaluate the functional status of the pregnant rats in each group during the CEUS, changes in heart rate were recorded, as shown in Figure 1.

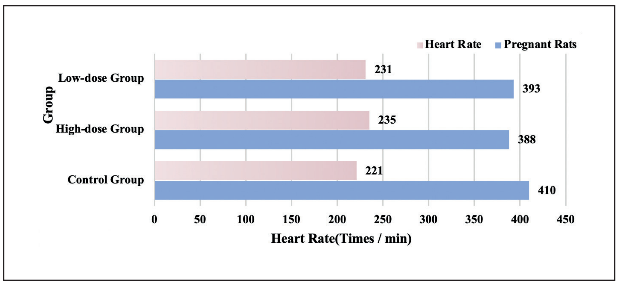

Figure 1: Comparison of the heart rates of the rats in each group.

Figure 1 shows that although the heart rates of the mothers and foetuses in the three groups decreased significantly (P=0.045, P=0.041), there were no significant differences between the three groups (P>0.05).

The changes in heart rate reflect the functional state of the vagus and sympathetic nervous system and can also be used as one of the indexes to evaluate the functional state of the organism. It was found that the heart rates of the rats in the control group was lower than that in the control group, but there was no significant difference between the three groups of contrast medium groups. This phenomenon is not caused by an injection of contrast agent microbubbles; rather, it may be caused by the stimulation of the vagus nerve and the heart rate by the rapid intravenous injection of a hypothermic solution. Alternatively, it may be related to the increase of volume load caused by the rapid injection.

The changes in the placental ultrastructure of the rats were observed by SonoVueTM. There were no significant changes in the placental ultrastructures of the rats in the low-dose group, suggesting that the steady-state cavitation reaction may be related to the microstructure of the contrast medium. The smaller the concentration of microbubbles, the fewer the total number of microbubbles in the placental volume of the rats. In addition, the total number of microbubbles with the same frequency of resonance characteristics and sound waves decreased, and the number of microbubbles flowing into the active state of the human body decreased, meaning that the damage to nearby trophoblast cells would be reduced. In the high-dose group, only the ultrastructural changes of the placental syncytial cytoplasm were observed immediately after CEUS, which may be related to the steady-state cavitation reaction of the contrast medium.

In steady-state cavitation, microbubbles exhibit short amplitude waves in the frequency of the sound field. Spherical pulsation produces a type of micro-acoustic current, which damages single trophoblast cells in the nearby subcellular organelles. In the high-dose group, no pathological changes such as nuclear fragmentation, nucleolysis or cell necrosis were observed. It was assumed that there were no obvious transient cavitations in the high-dose group.

Five days after CEUS, samples of placental tissue were taken, which showed that the basement membrane had become slightly thicker. Considering that the ultrastructural changes of trophoblast at high doses are probably salvage damage, the system seems to have been actively repaired. However, due to the small number of samples collected in this group of experiments, more data are needed to support this hypothesis.

Comparison of placental hemodynamic parameters of pregnant rats in each group ($\bar{x}\pm s$)

In order to further analyse the role of SonoVue™ in CEUS, the placental haemodynamic parameters of pregnant rats were observed and compared, as shown in Figure 2.

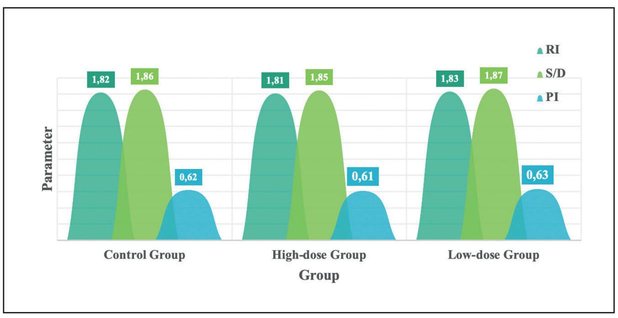

Figure 2: Comparison of the placental haemodynamic parameters of the pregnant rats.

Figure 2 shows that there was no significant difference in RI, PI and S/D values of the uterine arteries between the three groups (P>0.05).

The placentas of pregnant rats in the control group were not perfused with contrast medium. However, the placentas of the rats in the high-dose group showed contrast developing from the maternal surface approximately six seconds after injection, and the arterial ducts of the placenta and their continuous branches were perfused in sequence.

The placental maternal surface vessels showed an umbrella-shaped uniform filling process after 15-26 s, which was the peak of the video intensity. Approximately two minutes after injection, the contrast medium began to diffuse, and six minutes after injection it had almost disappeared.

In the low-dose group, the contrast agent was perfused in sequence, but the filling video intensity was low, the duration was short, and the contrast agent diffused rapidly.

In the high-dose group and the low-dose group, no contrast agent perfusion was found in the umbilical vessels of the foetal rats.

Ultrastructural changes of placental tissue of pregnant rats after CEUS

In the blank group, the placental tissue of the 12 pregnant rats which were selected immediately after the CEUS showed that the placental barrier layers were clear. Additionally, the cells of the placental barrier in the low-dose group did not change significantly.

However, in the high-dose group, the number of lysosomes in the cytoplasm of the syncytiotrophoblasts increased and the endoplasmic reticuli grew up.

Although ultrastructural changes, such as glycogen precipitation and vacuoles in the cytoplasm of some syncytiotrophoblast cells were observed, no abnormal phenomena were found in the endothelial cells or the basement membranes. Half a day after CEUS, the ultrastructure of the placentas of the rats in the control group and the low-dose group had not changed significantly; only the basal membrane of the placentas in the high-dose group had become thicker.

EM features of the placentas in pregnant rats

In order to analyse the ultrastructural changes of the rats' placentas after CEUS, any characteristic changes in the placentas detected under an EM were recorded, as shown in Figure 3.

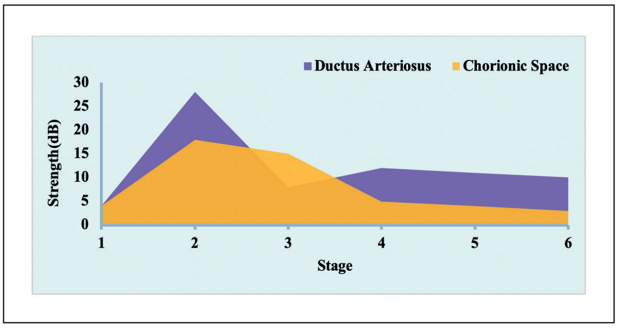

Figure 3: Placental features detected by electron microscope.

Figure 3 shows that the time-intensity curve of the arterial duct and villous space in an individual rat placenta is different. The curve shape of the arterial duct is wider, while the curve shape of the villous space is narrower. According to the time-intensity curve parameters of two different parts of rat placenta of ductus arteriosus was earlier than that of villous space at, and pit was earlier than that of villous space. The difference between the two parts was statistically significant (P<0.05). The Pi of the ductus arteriosus was significantly higher than that of the villous space (P<0.05). Additionally, the AUC of the ductus arteriosus was significantly larger than that of the villous space (P<0.05).

In conventional placental SonoVue™, microbubbles can only flow into the maternal surface of the placenta, not to the side. This indicates that the contrast agent itself cannot cross the placental barrier. CEUS can produce many biological reactions on target cells, and the cavitation effect can improve the permeability of the cell membrane. Ultrasound contrast agents can also change the permeability of other barriers during CEUS. Maternal blood circulation in the placenta can have a variety of effects on the growth and development of the foetus. The inflow of ultrasound contrast agent into the blood vessels of the foetus can reflect its blood supply. Trophoblast and vascular endothelial cells have similar properties as they can replace endothelial cells and form the inner layer of the vascular wall.

As gestation progresses, the connective tissue in the villi will become less and the cytotrophoblast will degenerate. Therefore, when the villi space diffuses into the maternal peripheral blood supply and into the villi tissue around it, CEUS will not increase the permeability of the placental barrier, and the contrast agent will not use the capillary walls of the foetal rats to flow into their circulatory systems. In this experiment, when using CPS imaging technology after injecting a high dose of microbubble contrast agent, the blood perfusion and sedimentation of the placental maternal surface can be clearly displayed, along with the villous arterioles and spiral arteries. However, low-dose contrast perfusion showed poor placental quality, and the disappearance of the contrast medium could not be clearly observed due to its short duration.

This is because the average diameter of the SonoVueTM microbubbles is 2.5 m, and the optimal frequency of resonance reflection is 4.5 MHz. Therefore, when higher frequency imaging is used, we must use more microbubbles to compensate. The sensitivity of microbubble imaging decreases because of its high sensitivity.

Comparison of time-intensity curves of different placentas in pregnant rats ($\bar{x}$±s)

In order to analyse the ultrastructural changes of pregnant rats at different times, the measured values of the time-intensity curve in different parts of the placentas of pregnant rats were compared, as shown in Figure 4.

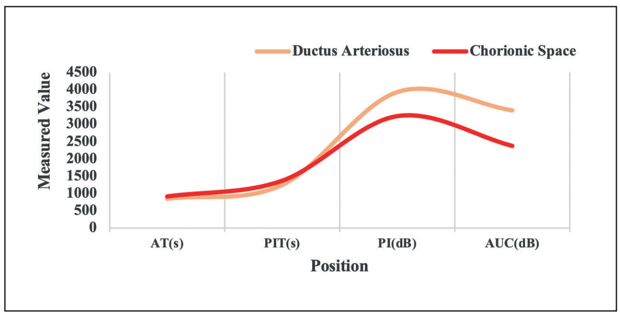

Figure 4: Comparison of time-intensity curves in different parts of the rat placentas.

Figure 4 shows that the shape of the time-intensity curve obtained in the two different parts of the rat placenta is relatively consistent, showing a rapid, steep ascending branch. After reaching the strength, there is a short plateau period before the curve slowly declines. The results show that the time-intensity curve of the region of interest (the placental ductus arteriosus) was wider than that of the chorionic space. The differences of AT, PIT, PI and AUC between the two parts were statistically significant (P<0.05), indicating that the quantitative time-intensity curve of CEUS can reflect the basic characteristics of uterine placental blood circulation in rats. The changing trends of the placental ductus arteriosus and chorionic spaces are very different. The tortuous shape of the ductus arteriosus was larger and the villous space was smaller. It is generally believed that the differences in curve shapes are due to the following three reasons:

- The faster the blood flow speed, the narrower the curve and the faster the dilution speed.
- The average flow time of the contrast medium can be regarded as the time when the contrast agent in the unit volume of blood vessels or components is completely replaced by fresh blood. The longer the average flow time, the smoother the curve.
- The larger the range of blood flow across the upstream and downstream of the sample, the larger the area under the curve. When the sample sizes are the same, there will be more contrast agent in the larger placental ductus arteriosus than in the villous parenchyma. Therefore, the amount of contrast medium in the upstream and downstream blood flow is much higher than in the villous parenchyma.

In addition, the large resistance (compared with the smaller resistance of the parenchymal vessels) means that the diffusion process of the contrast medium is significantly slower than it is in the parenchymal vessels. Therefore, the change curve of the placental artery catheter is more than that of the villus The substantial part is much wider. There was a linear relationship between AUC and local blood flow. The blood flow increased with the increase of AUC, which had the same characteristics as the blood circulation of the rat placenta. The blood flow in the villi is only a part of the blood flow of the ductus arteriosus, and its blood flow must be much lower than that of the ductus arteriosus. Therefore, its curve is much narrower.

Gross and light microscopic findings of placentas in three groups

In order to study the effect of SonoVue™ on CEUS, the gross and microscopic characteristics of the rat placentas in each group were recorded and compared, as shown in Table 1.

Group	General View	Under Low Power Microscope (*60)	Under High Power Microscope (*600)		
			Villous space	Villus morphology	Villous vessels
Control group	Dark Red, smooth surface, no abnormality	The structure of the fourth floor is clear	Villous space is wide	Villus morphology	Filling foetal rat red blood cells
High-dose Group	Tubercle, no abnormality	The structure of the fourth floor is clear	No narrowing was found in normal	No cell necrosis was found	No dilation, congestion or blood stasis was found
Low-dose Group	Tubercle, no abnormality	The structure of the fourth floor is clear	No narrowing was found in normal	No cell necrosis was found	No dilation, congestion or blood stasis was found

Table 1: Gross and light microscopic findings from the placentas in the three groups.

Comparison of blood lead level and placental tissue NO levels of pregnant rats in each group ($\bar{x}$±s)

In order to study the ultrastructural changes of placentas of pregnant rats in each group, the levels of blood lead and NO in the placental tissue were recorded and compared at the end of pregnancy, as shown in Table 2.

Group	Blood Lead/μmol $\cdot L^{-1}$	NO Level/μmol $\cdot L^{-1}$
Control Group	0.05±0.01	31.60±11.57
High-dose Group	1.26±0.25	132.03±17.52
Low-dose Group	0.60±0.10	98.35±19.36
P	<0.01	<0.01

Table 2: Comparison of blood lead and placental NO levels in pregnant rats.

Table 2 shows that the content of NO in placental tissue of pregnant rats in the two ultrasound contrast agent groups was higher than in the control group (P<0.01). The content of NO in the high-dose group was the highest. NO can regulate haemodynamics during pregnancy to meet the needs of the foetus so that the placenta has enough blood supply to ensure foetal nutrition and oxygen supply. In vitro experiments also showed that an appropriate amount of NO plays an important role in maintaining the development and differentiation of first-trimester embryos. MMP-9 is a rate-limiting enzyme in the invasion of embryonic trophoblast cells. It is one of the most important matrix metalloproteinases (MMPs) secreted by trophoblast cells. HJ plays an important role in trophoblast implantation, placental formation and other reproductive processes. Under physiological conditions, placental trophoblast cells can produce a certain amount of NO by regulating the expression and activity of MMP-9. Trophoblasts can invade the uterus, and NO can induce the production and secretion of MMPs. In recent years, CEUS has been widely used in the diagnosis of gynaecological diseases and has achieved favourable results. However, in clinical application, whether ultrasound irradiation combined with contrast agent microbubbles can penetrate the placenta barrier through cavitation. The effect on the foetus has not been reported. In order to evaluate the effect of CEUS on placental ultrastructure, different doses of CEUS were used in this study. As a barrier between mother and infant, the placental barrier has a more rigorous structure than the blood-brain barrier or the capillaries. It is a selective osmotic membrane through which nutrients, metabolites and antibody proteins can pass through. However, larger and more harmful molecules will usually find it difficult to pass through. The placental barrier consists of four layers, which makes the placental barrier thicker and more robust than the rest of the body. Therefore, the cavitation reaction of ultrasound microbubbles cannot improve the permeability of the placental barrier, and its specific mechanism needs to be confirmed by more in-depth and high-quality experimental data.

Conclusion

This paper describes the definition and role of SonoVue™ contrast agent and CEUS, briefly analyses the influence of placental shape on pregnancy and discusses the related role of ultrastructure. It has also shown that a low dose of SonoVue™ can play an important role in the observation of CEUS in late pregnant rats. In this study, 36 pregnant rats were randomly divided into three groups: a blank control group, a high-dose contrast medium group and a low-dose contrast medium group. After injecting contrast medium for approximately six seconds, the contrast in the placentas of the high-dose group was shown to gradually develop from the maternal surface, before spreading to the arterial ducts and their respective branches. After 15 to 26 seconds, the placental maternal surface vessels were evenly distributed in an umbrella shape, which was the maximum intensity of the video. There were no obvious pathological changes in the ultrastructure of the placental tissue between the control group and the low-dose group. It can be assumed, therefore, that it is both safe and feasible to use a certain amount of SonoVue™ contrast agent to visualise the placentas of rats in the third trimester of pregnancy. This study has significance for the ultrastructural effect of SonoVue™ in obstetric clinical research.

Although many scholars have made great advances in the study of CEUS, at the same time, and given the limitations of this study, we hope that CEUS should also consider other issues that are easy to be ignored while meeting the needs, so as to better improve the effectiveness of contrast agents and CEUS technology and to use them more widely. If the operational method of CEUS can be further applied in animal experiments and clinical practice, it will have far-reaching and significant consequences for future medical research.

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