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Contrast-enhanced ultrasonography of the placental barrier; the protective umbrella of the fetus during pregnancy

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Abstract

Aims: Ultrasonography is the preferred technique to evaluate the status of maternal and fetal health during pregnancy. Non-obstetric acute or chronic conditions occurring during pregnancy must be diagnosed as early as possible to permit timely and necessary treatment for the sake of maternal and fetal health. The purpose of this study was to evaluate the safety and value of contrast-enhanced ultrasonography (CEUS) during pregnancy. **Materials and methods:** This prospective study included 14 pregnant women requiring pregnancy termination and six healthy pregnant women. The 14 pregnant women requiring pregnancy termination underwent CEUS prior to surgery to investigate the pattern of contrast agent diffusion. The six healthy pregnant women did not undergo CEUS. The structure of placentae with and without contrast agent injection were also compared by light microscopy. **Results:** CEUS analysis failed to identify any signs of contrast agents in the umbilical cord blood and fetus. There were no obvious changes in the morphology of placentae with and without contrast agent injection under light microscope. CEUS identified the need for early treatment in one pregnant woman with an ovarian tumor. **Conclusions:** Due to the protective effect of the placental barrier on the fetus, CEUS during pregnancy may represent a safe form of imaging technology that can provide valuable information for the diagnosis of non-obstetric acute or chronic disorders and to guide the future treatment of pregnant women.

Keywords: Contrast-enhanced ultrasonography; SonoVue; pregnancy; placental barrier; fetus

Introduction

Non-obstetric acute or chronic disorders may occur during pregnancy. Such diseases must be diagnosed and treated as early as possible to preserve the health of the pregnant woman and fetus. Imaging techniques are important tools for diagnostic evaluation of acute and chronic diseases. Due to the use of radiation, however, computed tomography (CT) may cause miscarriage, fetal growth restriction, fetal malformation and neonatal mental retardation [1]. In the context of CT scans, iodinated contrast agents have been reported to pass the placental barrier [2]. Therefore, CT and other similar enhancement scanning technologies should be avoided in pregnant women unless there are strong clinical indications that the benefits of CT outweigh the risks. Magnetic resonance imaging (MRI) is a non-radiative technology that can fully penetrate and image soft tissues independent of the technician's experience. The major disadvantage of MRI is related to the prolonged time for acquisition; this may be uncomfortable for pregnant women. Although there are theoretical concerns, including the tissue heating and acoustic damage, there have been no reports of damage being incurred by the fetus in response to this form of evaluation [3]. The American College of Radiology does not recommend MRI during the first trimester of pregnancy [4]. Gadolinium-based contrast agent (GBCA) is one of the most-commonly used contrast agents for contrast-enhanced MRI. GBCA has a low incidence of anaphylaxis and is considered safer

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than iodinated contrast agents [5]. However, discussions concerning the safety of GBCA have intensified following reports of gadolinium residues accumulating in the brain. Even a single dose of GBCA can lead to the long-term deposition of gadolinium [6] and the long-term effects of gadolinium deposition in the brain structure are unclear. A previous study reported that GBCA can cross the placenta and enter the fetal circulation and amniotic fluid [7]. A previous animal study also showed that high concentrations of GBCA could impair neurological and motor function in fetal rats [8], thus suggesting that it is necessary to further investigate whether GBCA can exert adverse effects on the central nervous system of the fetus. Therefore, the use of GBCA in contrast-enhanced MRI during pregnancy remains a controversial issue.

Ultrasonography (US) is the most-commonly used diagnostic tool during pregnancy. As long as the time and parameters of insonation are in accordance with the ALARA principle, meaning that they are "as low as reasonably achievable", the US technique is not anticipated to affect the health of the fetus or pregnant woman. Contrast-enhanced ultrasonography (CEUS) provides important information when conventional US does not satisfy diagnostic needs. SonoVue is characterized by high molecular weight, low solubility, good stability and high echogenicity, and has been used widely in clinical diagnosis [9]. However, there are very limited data available at present relating to the safety of CEUS during pregnancy. The application of CEUS during pregnancy has not been officially approved by leading societies for obstetrics and ultrasound due to concerns relating to the potential adverse effects of contrast agents on the fetus. The World Federation of Ultrasound Medicine and Biology (WFUMB), in cooperation with the European Federation of Ultrasound Medicine and Biology (EFSUMB), outlined in the 2020 Guidelines for Liver CEUS [10] that no adverse events have been reported in recent studies relating to the utility of CEUS for assessing non-obstetric conditions in pregnant women [11-13].

The placenta is a temporary organ that is responsible for the exchange of material between the fetus and mother. There are two blood circulation pathways in the placenta. The maternal blood circulation pathway emanates from the branches of the uterine artery and flows into the uterine vein through the spiral artery and blood pool in the villous space. The branches of the umbilical vessels run radially from the site of attachment of the umbilical cord to the surrounding areas. The fetal blood circulation pathway emanates from the umbilical artery and flows into the umbilical vein through the capillaries in the villi. In the placental lobule, the villous capillaries carry the fetal blood and the maternal blood flows into the villous space. There is no direct communication between the maternal blood and fetal blood. The vascular-syncytial membrane (VSM) is the main site of exchange in the placenta and is composed of syncytiotrophoblasts, the syncytiotrophoblast basement membrane, chorionic stroma, the capillary basement membrane and capillary endothelial cells. The VSM has strict selectivity for the entry and exit of substances [14] in a manner that is similar to the blood-brain barrier and can protect the fetus from harmful substances to a certain extent, except that the placental barrier is thicker and denser than other barriers in the body. Previous studies have shown that the contrast agents used in US can increase the permeability of the blood-brain barrier [15-17]. However, Hua et al [18] reported that contrast agents do not alter the permeability of the placental barrier in pregnant rats. Further clinical trials are now needed to prove that SonoVue is safe to use in pregnant women.

In this study, we investigated the protective effects of the placental barrier on the fetus by analyzing CEUS images and comparing the morphology of delivery placentae with and without contrast agent injection.

Materials and methods

Patient characteristics

This prospective study comprised 20 pregnant women undergoing prenatal examinations at a local Hospital between September 2020 and January 2021. Fourteen pregnant women requiring pregnancy termination (ten with severe fetal abnormalities or fetal chromosomal abnormalities, two with scar pregnancies, one with a cornual pregnancy and one with an ovarian tumor) underwent CEUS before surgery. The purposes of preoperative CEUS were to observe the diffusion of contrast agent and to assess 13 cases of adhesion between the gestational sac or placenta and the myometrium and the nature of an ovarian tumor in one case. To observe whether the placentae exhibited structural changes under the light microscope after contrast agent injection, we selected six cases with placentae formation from 14 patients undergoing CEUS and compared them with six cases of placentae in normal pregnancy without CEUS.

We informed all participants about the safety of CEUS and the purpose of postpartum placental pathology examination and obtained their informed consent. The study was approved by the local Ethics Committee.

Contrast-enhanced ultrasonography

All US was performed by the same sonographer with more than 10 years of experience in obstetric scanning and rich experience in CEUS in gynecology. The pregnant women were examined by conventional US using

a 3.5-5.0 MHz transabdominal transducer (Hitachi Aloka ARIETTA 70) or a transvaginal transducer (Toshiba Aplio i800). A nurse established the intravenous pathway for the pregnant woman. The contrast agent, SonoVue (Bracco, Milan, Italy), was prepared by dissolving the freeze-dried powder in 5 mL of 0.9% [wt/vol] sodium chloride solution; for each patient, we injected 2.4 mL.

The sonographer identified the region where the umbilical cord joined the placenta, fixed the probe and then entered contrast mode at a low mechanical index (MI). The nurse quickly injected the prepared SonoVue into the elbow vein of the pregnant woman and flushed the tube with 5 to 10 mL of normal saline. The sonographer observed and recorded the diffusion of the contrast agent in the myometrium, placenta and fetus, and then scanned the entire placenta. The CEUS process continued until the contrast agent had disappeared completely. The nurse observed whether the pregnant women had adverse reactions.

Pathological analysis of placentae

The pregnant women received an induced labor after undergoing CEUS examination. After the placentae were delivered, placental specimens were taken from the region where the umbilical cord joined the placenta. Tissues were fixed with 4% [wt/vol] paraformaldehyde for 24 hours, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Cross-sections were then examined under a light microscope (magnification, 200×). The number of villi, the number of villous interstitial vessels, and changes in the villous interstitium and blood vessels in the placentae with and without contrast agent injection were then compared.

Results

Contrast-enhanced ultrasonography

The mean maternal age and gestational age at the time of CEUS were 27.2±2.9 years and 19.8 ± 9.8 weeks, respectively (Table I). Approximately 9.3±2.7 seconds after injection of the contrast agent, the uterine serosa, uterine basal decidual vessels, placental base and maternal placental lobules, were perfused until the placenta was fully filled and showed homogeneous high enhancement (fig 1). The contrast agent started to fade after approximately 6 - 7 minutes and disappeared completely after approximately 10–14 minutes. No contrast agent was observed in the umbilical cord blood and fetus. For cases of scar pregnancy with adhesion suspected by conventional US, CEUS revealed a clear demarcation between the gestational sac and the myometrium (fig 2). One case with an ovarian tumor presented with rapid heterogeneous high enhancement in CEUS (fig 3). No intrauterine residue was detected in any of the pregnant women after surgery. There were no technical difficulties

Table I. Pregnant women information

Patient	Age (years old)	Gestational age (weeks)	Causes of terminated pregnancy
#1	28	25	diaphragmatic hernia
#2	30	25^{+4}	endocardial cushion defect
#3	27	23^{+1}	cystic renal dysplasia
#4	25	33^{+2}	agenesis of corpus callosum
#5	32	21^{+2}	dyschondroplasia, strephenopodia
#6	31	25^{+3}	eyeball dysplasia with cataract
#7	28	19^{+6}	Down syndrome
#8	29	12^{+4}	exencephaly
#9	28	24^{+2}	cleft lip and palate, tetralogy of Fallot
#10	27	37	total anomalous pulmonary venous connection
#11	22	9^{+6}	scar pregnancy
#12	23	7^{+3}	scar pregnancy
#13	27	7^{+2}	cornual pregnancy
#14	24	6^{+5}	pregnancy with ovarian tumor

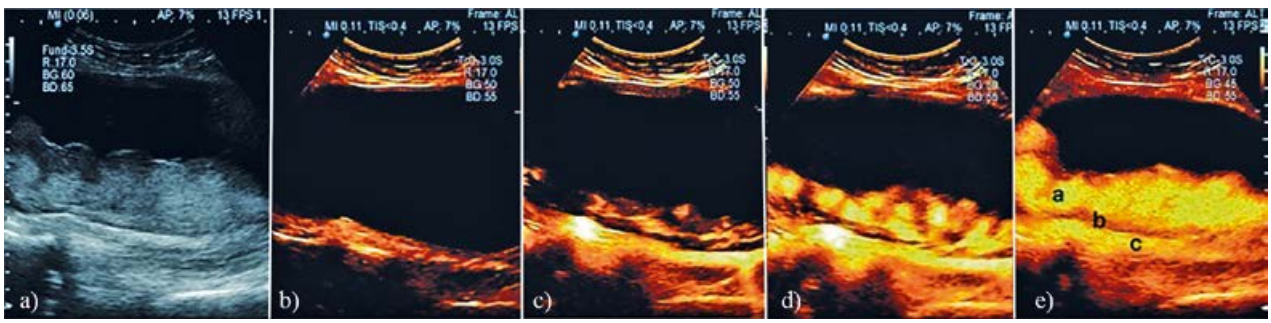

Fig 1. The contrast agent gradually spread from the maternal side to the fetal side until the entire placenta was labelled: a) the placenta was located in the posterior wall; b) the myometrium began to perfuse; c) the placental base began to perfuse; d) the contrast agent was filled into the placental lobules; e) homogeneous high enhancement throughout the placenta.

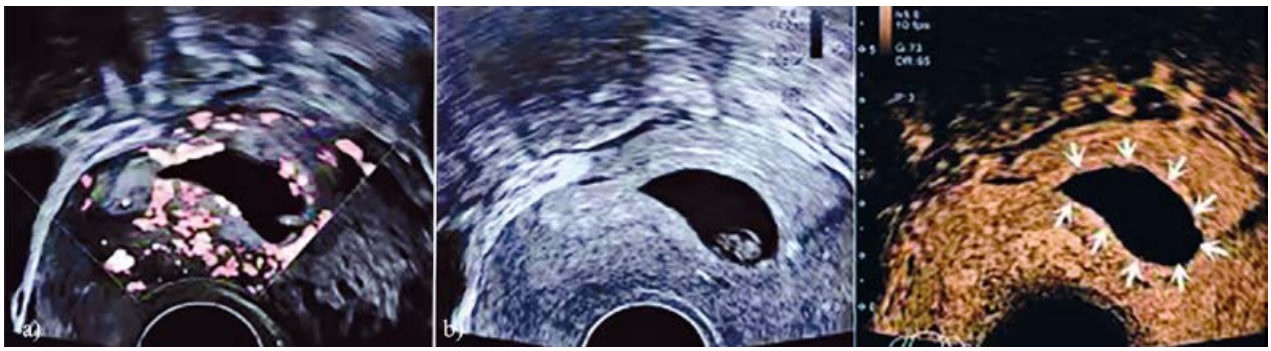

Fig 2. A 23-year-old female at around 7 gestational weeks with a history of cesarean section: a) the gestational sac was located within a scar in the lower segment of the anterior uterine wall and there was a moderate abundance of blood flow between the gestational sac and the cesarean section scar; b) the chorionic sac showed homogeneous annular enhancement (the white arrows) although there was no contrast agent in the embryo.

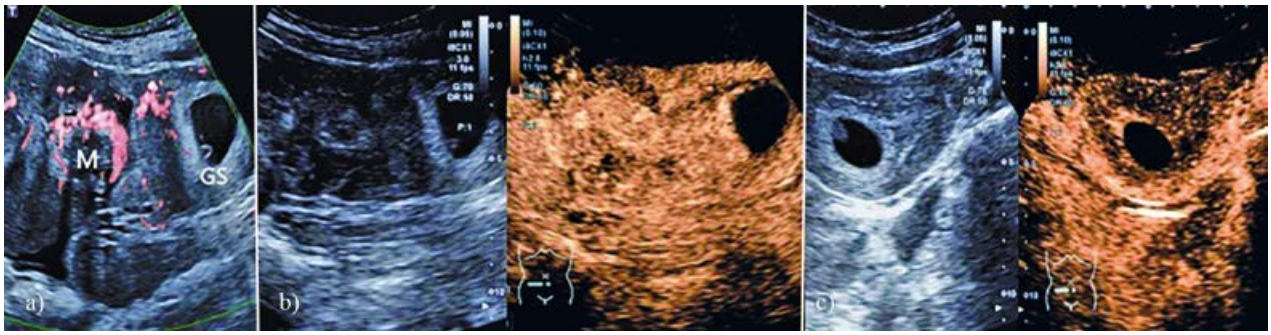

Fig 3. A 24-year-old female at around 6 gestational weeks with an ovarian tumor: a) a hypoechoic ovarian mass with hypervascularization; b) the solid tumor showed heterogeneous high enhancement; c) the chorionic sac showed homogeneous annular enhancement, while no contrast agent was observed in the embryo. M, the ovarian mass; GS, the gestational sac.

during CEUS and no adverse reactions, such as nausea, abdominal pain, headache, itching, rash or allergy in any of the pregnant women.

Pathological results

The placentae were delivered approximately 24 to 48 hours after CEUS examination. Under light microscopy, numerous placental villi could be seen in the maternal villous space of the placentae without contrast agent injection. Cytotrophoblast cells were scattered throughout the area. Syncytiotrophoblasts surrounded the periphery of the villi. The small nuclei were stained dark blue, whereas chromatin was stained light red and distributed throughout the nuclei. There were 2–10 vascular lumens in each villus, in which maternal red blood cells were also observed. Some of the villi showed interstitial fibrosis. The morphology of the placental villi injected with contrast agent were normal and no vacuoles were detected within the syncytiotrophoblasts. There was no necrosis, and no other abnormalities compared with the placentae without contrast agent injection. There was no fibrinoid necrosis of the placenta, and no apparent signs of congestion in the vascular lumens of villi in any of the cases with and without contrast agent injection (fig 4).

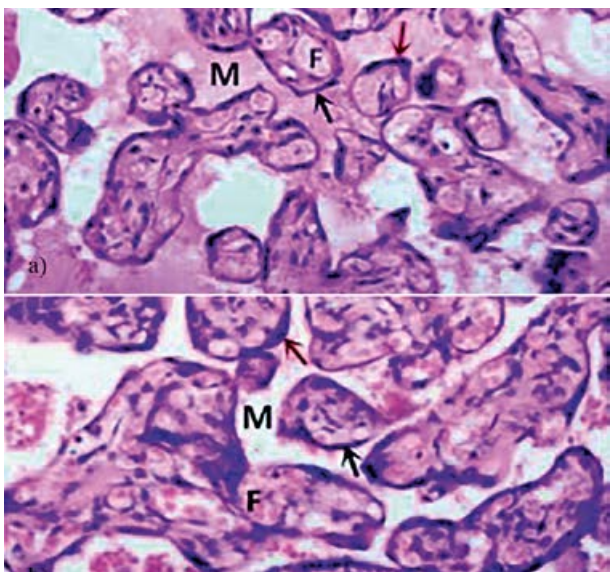

Fig 4. Histopathological correlates in placental tissue (HE staining, magnification, 200×): (a) a placenta injected with ultrasound contrast agent; (b) a placenta that had not been injected ultrasound contrast agent. Black arrows, the placental villi, red arrows, the syncytiotrophoblast; M, the placental villus space; F, the vascular lumen.

Discussion

SonoVue is a micron-scale contrast agent. Microbubbles can reach and be dispersed in various tissues and organs through the pulmonary circulation and systemic capillaries [19]. Approximately 99% of microbubbles in the pulmonary capillary bed can pass through capillaries into the alveoli and eventually out through the airways, thus avoiding accumulation in the human body [20]. The width of the transtrophoblastic channels in the placental barrier is approximately 20 nm [21] while the diameter of ultrasonic microbubbles exceeds 1 μm. Under normal circumstances, contrast agents cannot cross the placental barrier into the fetal circulation. The placenta begins to develop around the 9th week of embryo development, an event that can be observed by US. Placental development is completed by the 10th to 12th week of embryo development. As structural units of the placenta, the villi begin to grow after 18 to 20 days of embryonic development and subsequently form the chorionic sac on the surface of the embryo [22]. As shown in figure 2, CEUS revealed the homogeneous annular uptake of the contrast agent by the chorionic sac, while no contrast agent was observed in the embryo. The findings of this case imply that before the placenta is visible by US, syncytiotrophoblasts on the surface of villi have already protected the embryo [23]. In a previous study, Enders et al suggested that the placenta undergoes substantial structural changes during pregnancy [24]. Therefore, the role of the placental barrier in embryo development should be considered at every stage of pregnancy. Our results showed that no microbubbles of contrast agent were detected in the fetal umbilical circulation in the first, second or third trimesters of pregnancy, thus confirming the results described in previous publications [25,26].

Previous studies involving the use of US contrast agents during pregnancy selected women who did not intend to maintain their pregnancy as research objects and included the analysis of uterine placental blood flow [27], the diagnosis of cesarean scar pregnancy [28], the evaluation of invasive placenta percreta [25] and analysis of placental spiral artery plugging [29]. No adverse effects were reported after the use of contrast agent in any of these previous studies. Until now, no previous studies of placental pathology have been reported in humans following CEUS. In the present study, we tried to perform a preliminary study of the placental pathology of pregnant women by light microscopy. Due to the limited sample size, this study was limited to the pathological comparison of placentae by visual observation. Our study showed there was no obvious abnormalities under light microscopy when compared between placentae with and without contrast agent injection. With the increased use of CEUS in clinical settings, many investigators have reported cavitation effects caused by microbubbles with increased levels of ultrasound output energy [30]. We hypothesized that if the cavitation effect induced by microbubbles occurred in the intervillous space, then this would lead to syncytial trophoblast necrosis and villous vascular lumen congestion. We observed no such pathological changes in the placentae injected with contrast agent when examined by light microscopy. Micro-damage caused by CEUS may not be detectable under light microscopy and may require further analysis by electron microscopy. Other studies have described the safe use of placental CEUS in non-human primates. For example, Roberts et al reported that the use of CEUS did not cause placental structure damage in macaques [31]. Further studies are needed in the future to determine whether the use of CEUS causes micro-damage to the human placenta.

Maternal obesity, a deep placental position, fetal occlusion and other factors, are known to affect the results of conventional US. The lack of good contrast resolution between the placenta and myometrium is also known to limit its diagnostic value, whereas CEUS can judge the relationship between the placenta and the myometrium by revealing tissue perfusion. Consequently, CEUS has significant potential for the evaluation of placental disorders [25]. In addition, CEUS is instrumental for improving the management of patients. As we exemplified in figure 3, a pregnant woman was found to have a hypervascular mass in her right ovary on US. CEUS showed uneven and high enhancement of solid tumor, which occurred earlier than the uterus, suggesting that the tumor had partial necrosis, and the possibility of malignant transformation could not be ruled out. Considering the risks of torsion, rupture and malignant transformation of such a large tumor, the gynecologist recommended surgical removal of the ovarian tumor as early as possible. The final pathological results confirmed a diagnosis of ovarian fibroma. Recently published cases have also demonstrated the potential diagnostic value of CEUS for the evaluation of pregnant women with liver, kidney, or other non-obstetric disorders [11-13]. CEUS may represent a better choice when conventional US cannot meet diagnostic needs; that is, it could obtain sufficient information and avoid a delay in the diagnosis and treatment of diseases caused by pregnancy.

We acknowledge that this study has some limitations that need to be considered. Single-center experience, limited number of cases, and lack of interobserver represent main limitations. Due to the induced labor in pregnant women generally taking about 24 to 48 hours, the placenta cannot be immediately dissected for pathologi-

cal examination after the contrast agent injection as in animal experiments. All of the women in the study who underwent CEUS required the termination of pregnancy, so it was not possible to track whether the contrast agent would have long-term adverse effects on the fetus. Recent studies of CEUS in non-obstetric diseases during pregnancy have shown that pregnant women who were identified as having benign lesions by CEUS, chose to continue regular pregnancy management and subsequently delivered healthy newborns [11-13], thus suggesting that CEUS has good prospects in terms of safety and clinical value. Larger prospective multicenter studies are required to confirm the safety and clinical value of CEUS during pregnancy.

Conclusion

The results of our study demonstrated that ultrasonic microbubbles did not cause cavitation effects when applied with a low mechanical index and at a diagnostic dose. Due to the protective effect of the placental barrier on the fetus, ultrasonic microbubbles were unable to enter the fetus. CEUS is a safe and valuable imaging technology for the diagnosis of non-obstetric acute and chronic disorders during pregnancy.

Conflicts of interest: none.

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