—**Original**—

Determination of Blastocyst Implantation Site in Spontaneous Pregnancies Using Three-Dimensional Transvaginal Ultrasound

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Abstract

Three-dimensional transvaginal ultrasound was used to determine the location of early gestational sacs (E-GSs) and, as a result, the physiological implantation sites of human blastocysts. We examined 138 patients who were found by three-dimensional transvaginal ultrasound to have a GS with an inner diameter of 3 to 6 mm (E-GS). The uterine cavity was divided into three parts: upper, middle, and lower regions. The upper region was subdivided into the right, middle, and left areas, and the middle region was subdivided into the right and left areas. Thus, overall 6 areas were designed and the frequency of the E-GS detection in each area was evaluated. Of the 138 patients, 123 (89.1%) had E-GSs detected in the upper region, which was found to be the most frequent region. When the frequency of E-GS detection among the upper three areas was compared, the right and left upper areas had a higher frequency than the middle upper area. As to the miscarriage rate, patients with E-GSs detected in the upper region had a significantly lower rate than those in the middle and lower regions ($p<0.05$). The endometrium suitable for human blastocyst implantation under physiological conditions is at the uterine fundus, especially near the uterotubal junction.

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Key words: implantation site, three-dimensional transvaginal ultrasound, early gestational sac (E-GS), blastocyst

Introduction

Little has been determined regarding the human blastocyst implantation site in the uterine cavity. It has been reported that the physiological implantation site of a blastocyst is on the endometrium near the center of the fundus uteri.[1–4] However, since these reports are based on surgically removed uteri or endometrial curettage specimens and few actual cases, many related issues remain to be clarified. Presently, a gestational sac (GS) or an embryo in the uterine cavity can be observed easily from the early stages of pregnancy using transvaginal ultrasound.[5–7] However, with the two-dimensional ultrasound examination currently in general use, it is difficult to identify the exact location of a GS in the uterine cavity due to the limitations of the scan method.

In this study, we used a three-dimensional transvaginal ultrasound, which allows observation of the entire uterine cavity in a frontal view, to assess

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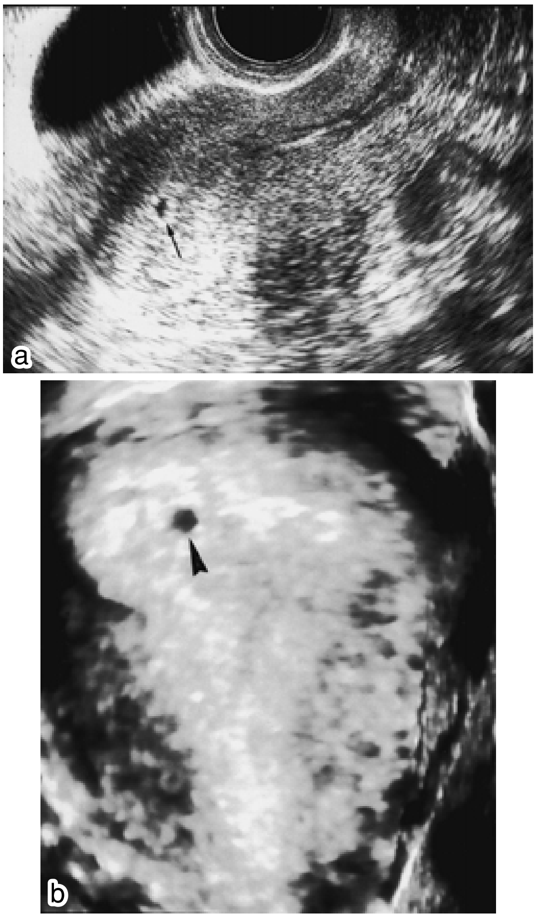

Fig. 1 Detection of GS (arrow) with two-dimensional ultrasound (a), and the same GS (arrowhead) with three-dimensional ultrasound (b)

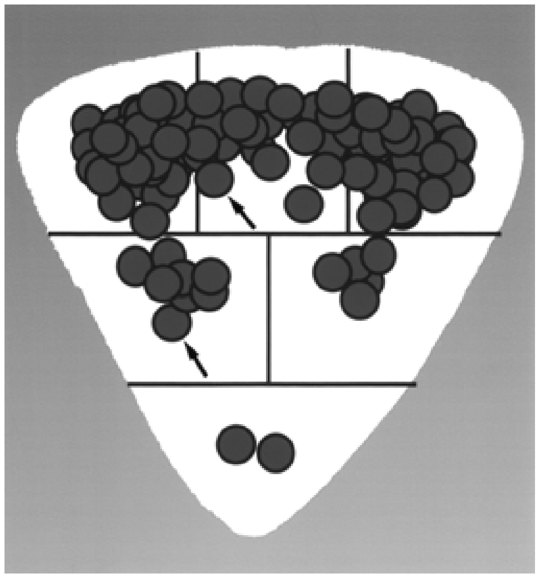

Fig. 2 The location of E-GSs (arrows) was determined in a frontal view of the uterus with three-dimensional transvaginal ultrasound. The uterine cavity was divided into three parts: upper, middle, and lower regions. The upper region was subdivided into the right, middle, and left areas, and the middle region was subdivided into the right and left areas. Thus, overall 6 areas were designed.

the location of GSs. This method allowed us to determine the location of early GSs (E-GSs) and, as a result, identify the physiological implantation sites of human blastocysts.

Subjects and Methods

This study consisted of 138 patients who visited the outpatient clinic at Nippon Medical School Second Hospital's Department of Obstetrics and Gynecology between April, 2000 and June, 2002 and were found by transvaginal ultrasound to have a GS with an inner diameter of 3 to 6 mm (E-GS) (**Fig. 1a**). The inner diameter of GS was defined as the longest diameter of the hypoechoic cystic region surrounded by the hyperechoic ring. All patients had a morphologically normal uterus and no history of cesarean section. With three-dimensional transvaginal ultrasound, the location of E-GSs was determined in a frontal view of the uterus (**Fig. 1 b**). The uterine cavity was divided into three parts: upper, middle, and lower regions. Furthermore, the upper region was subdivided into the right, middle, and left areas, and the middle region was subdivided into the right and left areas. Thus, overall 6 areas were designed (**Fig. 2**). The frequency of E-GS detection in each area was evaluated. When the GS was located on the borderline, it was classified in the area in which more than half of the GS resided.

Three-dimensional transvaginal ultrasound was performed after informed consent was obtained from the pregnant women. The ultrasound units used were a Sonovista-MSC (Mochida, Tokyo, Japan) and a Voluson 530 D (Medison-Japan, Korea) with 5.0, 6.0, and 7.5 MHz transvaginal probes. In GS search ability, no differences were recognized between the ultrasound units or among the probes that we used in this study. The statistical analyses were performed using the chi-square test. The statistical significance level was set at $p<0.05$.

Table 1 Frequency of the E-GS detection in each area and the miscarriage rate

Location	Upper region			Middle region		Lower region
	Left	Middle	Right	Left	Right	
No. of cases (n = 138)	54	22	47	5	8	2
No. of miscarriages (n = 17)	5	1	3	2	4	2
Miscarriage rate (%)	7.3* (n = 123)			53.3 (n = 15)		

*p < 0.05 vs. middle and lower regions (chi-square test)

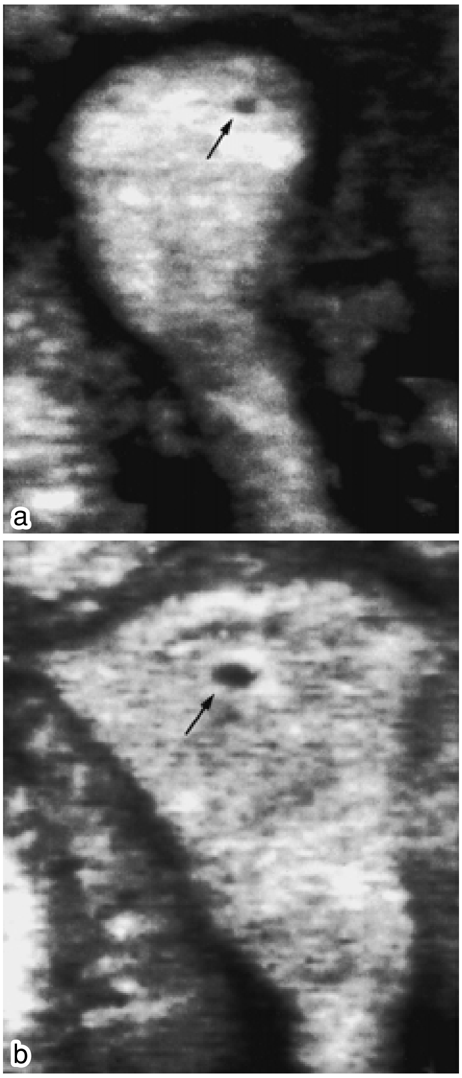

Fig. 3 Detection of GS (arrow) in the left upper area (a), and middle upper area (b).

Results

Table 1 shows the frequency of E-GS detection in each area and the miscarriage rate. In 123 of the 138 cases (89.1%), E-GSs were detected in the upper region (**Fig. 3 a, b**), which was found to be the most frequent region; 13 cases (9.4%) had E-GSs in the middle region (**Fig. 4**) and 2 cases (1.4%) in the lower region (**Fig. 5**). When the frequency of E-GS detection among the upper three areas was compared, the right and left upper areas had a higher frequency than the middle upper area. There were 17 cases that had miscarriages by the twelfth week of gestation. As to the miscarriage rate, patients with E-GSs detected in the upper region had a significantly lower rate than those in the middle and lower regions ($p<0.05$).

Fig. 4 Detection of GS (arrow) in the right middle area.

Discussion

The detailed processes underlying human blastocyst implantation are still unclear, but it is believed that the process would parallel that seen in the implantation of other mammalian blastocysts. The histological demonstrations by Hertig et al.[2-4]

Fig. 5　Detection of GS (arrow) in the lower region.

and Heuser et al.[1] regarding the implantations of macaque monkey and human blastocysts in the endometrium are well known. Ramsey et al.[8] used macaque monkeys, and Hendrickx et al.[9] used baboons to observe the implantation site of blastocysts. When the uterine cavity was divided into the anterior and posterior walls, and upper and lower halves with left, central or right positions, 18 of the 22 implantations had a central position [9]. Hertig et al. examined the implantation sites of 7 cases with normal human blastocysts discovered in surgically removed uteri and reported that the blastocysts of 7.5～16.5 days were situated 3 to 21 mm from the fundus[4]. But the limitations of that study were the retrospective nature and small number of cases. At present, the implantation site of blastocysts *in vivo* must be estimated from the location of the very early GS by ultrasound. Usually, the size of the early GS that can be detected by transvaginal ultrasound is 3 to 4 mm in diameter, which corresponds to the 17 th to 20 th day after fertilization[5-7]. It can be assumed that there is no considerable difference in the distance between these early GS locations and the blastocyst implantation sites.

Kinoshita et al.[5] divided the vertical section of the uterine cavity into upper, middle, and lower regions and examined the detection sites of GSs of 2 to 4 mm in diameter. They found that the frequencies were 80.8% in the upper region (highest), 14.5% in the middle region and 4.6% in the lower region. Their results partially agree with our results. However, their observations were acquired by two-dimensional ultrasound. Therefore, the entire uterine cavity could not be observed and the study was not detailed. An important aspect of our study was the detailed examination of GSs in the lateral uterine sides in addition to their locations in longitudinal section and the provision of a schematic representation for E-GSs. The sizes of E-GSs in this study were from 3 to 6 mm in diameter. Therefore, it can be assumed that blastocyst implantation sites correspond with E-GS detection sites. GSs detectable with three-dimensional transvaginal ultrasound were more than 3 mm in diameter. GSs equal to or less than 6 mm in diameter develop at almost the same position, but grow towards the central portion of the uterine body gradually, so the GS locations and the blastocyst implantation sites are not the same. There is no considerable difference in the distance between our early GS locations and the blastocyst implantation sites. With the two-dimensional ultrasound currently in general use, it is difficult to identify the exact location of a GS, particularly around the uterotubal junction due to the limitations of the scan method. However, three-dimensional ultrasound allows observation of the entire uterine cavity in a frontal view of the uterus, and thus precise localization of GSs. Therefore, we used three-dimensional ultrasound to search for the correct location of GSs in this study.

Jinno et al.[10], using a laser blood-flowmeter, reported that in normal uteri the endometrial tissue blood flow (ETBF) was significantly higher in the fundus than in the other regions. Also, as a part of their study, they evaluated the detection site of GSs after 5 to 6 weeks of gestation and concluded that 85% were located in the fundus, which has a rich blood flow. Based on these results, it was suggested that the ability of blastocysts to implant and the endometrial tissue blood flow were strongly interrelated. However, some limitations apply to these findings; for example, the GSs evaluated were more than 15 mm in diameter and the GS location could not be determined precisely due to the use of two-dimensional ultrasound. Only one study reported by Baba et al.[11] observed GS location by three-dimensional ultrasound. In that study, the GS location was observed after an embryo was transferred into the uterine cavity at the fundal area during *in vitro* fertilization and embryo transfer (IVF-ET). They found that 84.4% of the GSs were detected at the fundal area where they were initially transferred and 9.4% were detected in the middle region. These results are similar to our findings, but their study used artificial pregnancies. Our findings also agree with the findings of many other studies showing that blastocysts implant at the fundal area far more often than elsewhere. However, no reports have been carried out using three-dimensional transvaginal ultrasound to study implantation sites in normal pregnancies and demonstrating that blastocyst implantation is more often near the uterotubal junction.

One of the important conditions for blastocysts to implant is that the endometrial tissue blood flow in that area is rich. Around the uterotubal junction, the oviductal branch from the ovarian artery and the ascending branch of the uterine artery are anastomosed, and blood flow is especially rich, even though the fundal area in general is quite rich. Therefore, it is suggested that blastocysts are apt to implant at the fundal area where endometrial tissue blood flow is rich, especially the endometrium near the uterotubal junction.

The report by Kinoshita et al.[5] and our current study suggest that endometrial tissue blood flow is potentially a significant factor in the high miscarriage rate in cases with the E-GS implanted in areas other than the fundus. When the GS is observed daily, it develops from near the uterotubal junction and fundus, gradually towards the central portion of the uterine body. Therefore, we can assume that after the blastocyst implants in the uterine fundus, especially near the uterotubal junction, the GS grows towards the central portion of the uterine body, accompanying the development of the chorionic villi. Because the GSs we examined in our study were limited to the very early stages, it is assumed that the GS location corresponds to the blastocyst implantation site. The endometrium suitable for human blastocyst implantation under physiological conditions is at the uterine fundus, especially near the uterotubal junction.

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