

Supplementary Materials

Supple 1.

Measurement of serum hCG concentration. In normal pregnancies, the hCG levels in the blood can be detected shortly after implantation begins. At 4 weeks and 0 days of pregnancy, the blood hCG level reaches 100 - 200 IU/L. At 5 weeks and 0 days, the blood hCG level increases exponentially to approximately 2,000 - 4,000 IU/L. In ectopic pregnancy, implantation of the fertilized egg occurs at sites other than the endometrium of the corpus uterus. Thus, hCG production and secretion are lower than those in normal pregnancy [8]. In most cases, the blood hCG level (discriminatory zone) is approximately 1,000 - 2,000 IU/L when the GS becomes detectable on ultrasound.

Transvaginal ultrasound imaging. An intrauterine GS image is detected at the late fourth week of gestation by transvaginal ultrasound imaging, and GS is detected in nearly 100% of cases at the early fifth week of gestation. If an intrauterine GS image is observed, ectopic pregnancies are ruled out, with the exception of simultaneous intrauterine and ectopic pregnancies (i.e., heterotopic pregnancy), which are thought to occur at a frequency of 1/30,000 [11]. However, in cases of pregnancies via *in vitro* fertilization, the frequency of simultaneous heterotopic pregnancies increases to 1-3% [12].

Wolffian and Mullerian ducts. In the early stages of organogenesis, male and female fetuses have two reproductive ducts (i.e., the Wolffian and Mullerian ducts) [14]. The Mullerian ducts develop outside the gonads, whereas the Wolffian ducts develop via invagination of the coelomic epithelium. Cranially, the Mullerian duct opens into a body cavity in a funnel-like fashion, and caudally, the Mullerian duct meets the opposite Mullerian duct. In the male fetus, testosterone produced by the Leydig cells of the testis stimulates the development of the Wolffian ducts. The Mullerian duct inhibitory substances made by Sertoli cells inhibit the development of the Mullerian ducts (tissues of the female reproductive tract) [15]. The Wolffian ducts remain in the fetus to form the main male reproductive tract [15]. In the female fetus, the head of the Mullerian tube forms with the fallopian tube tissue, and the left and right Mullerian tubes are closely fused downward. This fused portion forms the tissue of the uterine tube.

Methotrexate. Methotrexate is a drug used to treat inflammatory diseases, such as rheumatoid arthritis and psoriasis, and malignant tumors [16]. When methotrexate is injected in patients with ectopic pregnancies, it can terminate the pregnancy by inhibiting embryonic or fetal cell division.