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# [Communication] Histopathological findings of ectopic pregnancy in contraceptive-wearing women

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## Abstract

In a normal pregnancy, the egg is fertilized in the fallopian tube. Afterward, it moves into the uterus, where it implants into the uterine endometrium. However, if the fallopian tubes are narrow or blocked, the fertilized egg cannot move into the endometrium. In this situation, the fertilized egg implants in tissue other than the uterus, resulting in an ectopic pregnancy. In most cases of ectopic pregnancy, the fertilized egg implants in the left or right fallopian tube. It can also implant in tissues other than the fallopian tubes, such as the ovary. This paper presents our experience in treating a 37-year-old woman who had a tubal pregnancy despite wearing a contraceptive. Contrast-enhanced magnetic resonance imaging showed a gestational sac behind the right fallopian tube. Laparoscopic surgery was performed to resect the right fallopian tube. Pathological examination suggested that the ectopic pregnancy occurred at the organogenesis stage 9 weeks after fertilization. The pathological findings revealed subpopulations of cells from the ectoderm that were separated from other cells and more specifically formed spinal and ovarian structures. This paper presents valuable surgical pathological findings for understanding the differentiation into each tissue during the organogenesis stage.

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**Short Title:** Pathological findings of ectopic pregnancy

Ectopic pregnancy refers to the implantation of a fertilized egg in tissues other than the endometrium. It is classified into four types depending on the implantation site of the fertilized egg: fallopian tube pregnancy, interstitial pregnancy, cervical pregnancy, and ovarian pregnancy. Among them, fallopian tube pregnancy is the most common, accounting for more than 90% of all ectopic pregnancy cases. Studies have shown that ectopic pregnancies occur in approximately 0.5%–2% of all pregnancies. In particular, women who have pelvic organ inflammation, abdominal surgery, or a history of ectopic pregnancy have increased risk of ectopic pregnancy. In recent years, the incidence of ectopic pregnancy has been increasing, given the increase in the number of women suffering from sexually transmitted diseases such as chlamydia and those undergoing infertility treatments (in vitro fertilization/embryo transfer).

Fallopian tube pregnancy is frequently associated with pelvic inflammatory disease, which may lead to fallopian tube stenosis. This condition prevents the fertilized egg from moving toward the uterus, causing it to attach to the tube itself. The symptoms of fallopian tube pregnancy include abdominal pain and vaginal bleeding. The first diagnostic step is to confirm the pregnancy with beta-human chorionic gonadotropin ( $\beta$ -hCG) testing; next, transvaginal ultrasound is performed to determine the location of the pregnancy and fetal heartbeat <sup>[1][2]</sup>. **note 1** Uncomplicated ectopic pregnancies often resolve spontaneously and are usually difficult to diagnose. Laparoscopy or laparotomy can also be performed to visually confirm an ectopic pregnancy. These procedures are generally performed for women presenting signs of an acute abdomen and hypovolemic shock.

Patients with ectopic pregnancy are typically hemodynamically stable with low, declining  $\beta$ -hCG concentrations ( $<5000$  IU/L) <sup>[1][2]</sup>. **note 1** Fetuses from ectopic pregnancies may survive for several weeks. However, they will eventually die because no tissue outside the uterus can provide their blood supply. In many cases, the structures containing the fetus rupture after about 6 to 16 weeks, long before the fetus can survive. Complicated cases may involve tubal abortion or rupture, which can lead to intra-abdominal bleeding and shock. Tissue rupture in an ectopic pregnancy can cause severe bleeding and can be life-threatening to the mother. The later the rupture, the greater the blood loss and the higher the risk of death. However, if the ectopic pregnancy is treated before it ruptures, the risk of death is decreased. Although uncomplicated cases are treated conservatively (e.g., methotrexate or expectant management), complicated cases require surgical removal. In cases of abdominal pain in women of reproductive age, ruptured ectopic pregnancy should be ruled out.

## Case 1

In July 2022, a 37-year-old woman had an intrauterine device (IUD; FD-1, Fuji Latex Ltd., Tochigi, Japan) implanted in her uterus. She experienced menstruation in September 2022 and no menstruation for 7 weeks thereafter. In November 2022,

she visited a nearby obstetrics and gynecology clinic. Clinical presentation of ectopic pregnancy occurs at a mean of 7.2 weeks after the last normal menstrual period, with a range of 4 to 8 weeks [3]. Later presentations are more common in communities deprived of modern diagnostic ability. At that time, her urine hCG concentration was high. Thus, it was thought that she might be pregnant. However, ectopic pregnancy was suspected because ultrasound imaging did not reveal a gestational sac (GS) in the uterus. She did not experience intra-abdominal bleeding. Ectopic pregnancy should be suspected if the hCG level is  $\geq 2000$  IU/L and no GS is observed in the uterus. <sup>note 2</sup>

## Blood testing of serum hCG levels and other indexes

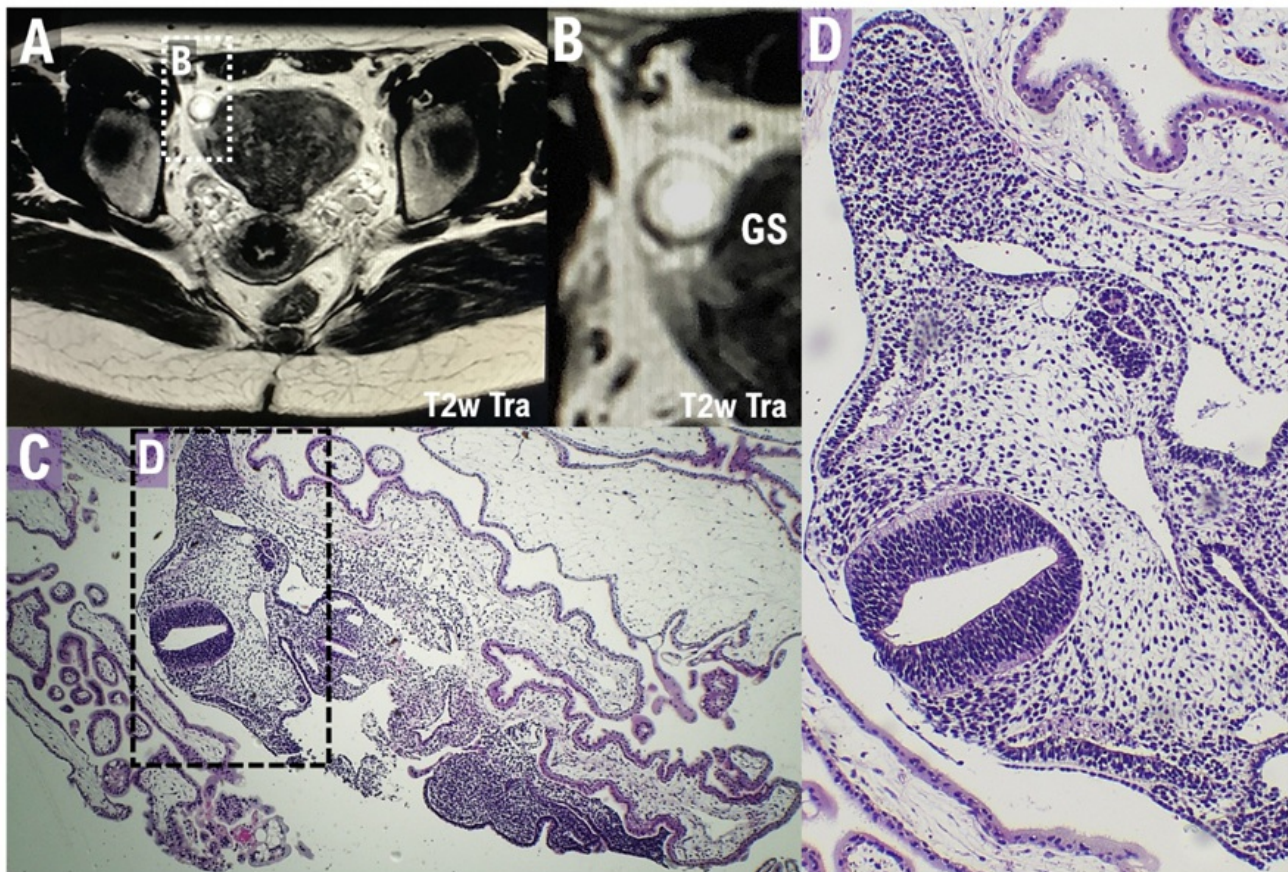
Changes in serum hCG over 48 h can be used to define the hCG ratio, which is calculated as follows: hCG ratio = hCG at 48 h/hCG at 0 h [4]. An hCG ratio of 0.87 (i.e., a 13% decrease in hCG over 48 h) has 93% sensitivity and 97% specificity for predicting a failing pregnancy of unknown location. When the patient first came to our general hospital, her blood test results showed a serum hCG level of 9316. Forty-eight hours later, her serum hCG level was 3773. Her hCG ratio after 48 h was 0.405 (a 59.5% reduction in serum hCG).

Her blood sugar level, serum iron level, and irregular antibodies were 106 mg/dL, 46 g/dL, and negative, respectively. She was tested for human immunodeficiency virus (HIV)-1/2, hepatitis B virus (HBV), hepatitis C virus (HCV), and *Treponema pallidum* (TP) to confirm the presence or absence of viral infections. Her results were as follows: HIV-1/2 (0.05 S/CO), HBV (HB antigen quantitative:  $<0.02$  IU/mL), HCV (HCV antibody: 0.1 S/CO), and TP qualitative antibody level (0.08 S/CO). However, in vivo inflammation was indicated because her C-reactive protein was high (0.29 mg/dL).

## Findings from magnetic resonance imaging (MRI) examination

Plain pelvic MRI and contrast-enhanced MRI were performed as the patient did not wish to continue her pregnancy. Ectopic pregnancy was suspected because MRI showed a cystic lesion with ring-shaped diffusion restriction and contrast enhancement in the right fallopian tube (Figure 1A, B). No structures suggestive of a GS were observed in the lumen of the uterus. Mild endometrial thickening was observed. A small amount of ascites was noted, but bloody ascites was negative. Contrast-enhanced MRI study revealed that the bilateral ovaries were not normal. The IUD was located in the isthmus of the uterus (Supplementary Figure 1A). Scarring from the Cesarean section was noted. Pregnancy in the right fallopian tube was suspected due to various findings on MRI examination (Figure 1A, B; Supplementary Figure 1B). A cyst was observed on the dorsal side of the left ovary, suggesting the development of an ovarian or paraovarian cyst (Supplementary Figure 1C).

Fig.1



**Figure 1. Diagnosis of ectopic pregnancy by contrast-enhanced MRI and histopathological examination.** **A.** The result described from contrast-enhanced MRI examination (T2w Tra) shows the possibility of ectopic pregnancy. **B.** The white dotted area shown in panel A is presented as a panel. **B.** The GS in the right fallopian tube is indicated by a white spherical structure. Ectopic pregnancy was suspected because MRI showed a cystic lesion with ring-shaped diffusion restriction and contrast enhancement in the right fallopian tube. **C.** A specimen of the resected right fallopian tube was used for pathological diagnosis. The white dotted area shown in panel D is presented as a panel. Histopathological examination reveals the histology of the developing fetus during the growth period.

## Tissue findings

Villi, trophoblasts, and embryos were observed in the fallopian tubes. Nucleated erythrocytes were observed in the blood vessels of the villi. Ectopic pregnancy was confirmed based on these tissue findings. Fetal malformations or malignancies were not suspected.

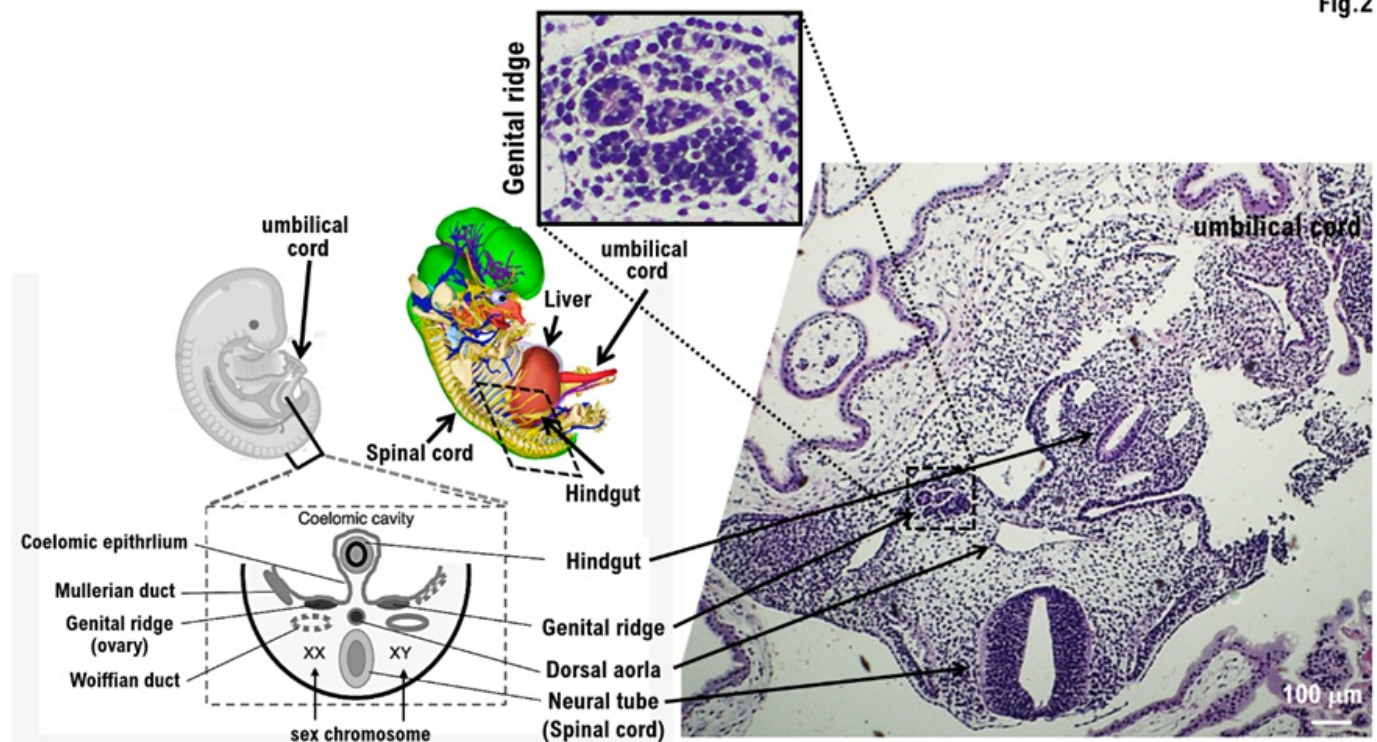
## Findings by pathological diagnosis

A specimen of the resected right fallopian tube was used for pathological diagnosis (Figure 1C, D; Figure 2). Villus-like structures and hemorrhages were observed. Based on the pathological examination findings, ectopic pregnancy occurred during the organogenesis period around the 9th week after fertilization (Figure 1C, D; Figure 2). There is no difference in the structure of the reproductive tract between males and females during the early stages of development. The Wolffian



and Müllerian tubes are formed early in development.<sup>note 3</sup> In females, the left and right Müllerian ducts fuse to form the uterus and the upper part of the vagina as the fetus develops. The unfused tissue becomes the fallopian tube, and the Wolffian ducts degenerate. If union is incomplete, the structure of the uterus and vagina will be malformed. On the other hand, in males, the Müllerian ducts degenerate, and Wolffian ducts are formed, primarily in the vas deferens.

Fig.2



**Figure 2. Structure of various tissues of the growing fetus via histopathological examination.** Histopathological examination reveals the structure of various tissues of the growing fetus after the 9th week of pregnancy. The images show the development of the neural tube (spinal cord), hindgut, and dorsal aorta. Pathological examination shows the development of the genital ridge. The genital ridge (or gonadal ridge) is the precursor to the gonads. Initially, the genital ridge consists mainly of mesenchyme and cells of underlying mesonephric origin. Once oogonia enter this area, they attempt to associate with these somatic cells. Pathological results suggest that the fetus may be female.

The pathological examination showed the development of the genital ridge (Figure 1C, D; Figure 2). The genital ridge (or gonadal ridge) is the precursor to the gonads. Initially, the genital ridge consists mainly of mesenchyme and cells of underlying mesonephric origin. Once oogonia enter this area, they attempt to associate with these somatic cells. The pathological results suggested that the fetus may be female. Development proceeds, and the oogonia become fully surrounded by a layer of cells (pre-granulosa cells). That is, the genital ridge is formed from the mesenchyme and coelomic epithelium at about 4 weeks of gestation and will ultimately provide somatic cells to the follicle (Figure 1C, D; Figure 2).

An ectopic pregnancy is when the fertilized egg implants somewhere other than the endometrium. Tubal pregnancies are the most common and account for approximately 95% of ectopic pregnancies. The risk factors for ectopic pregnancy are thought to include pelvic inflammation and fertility treatments. Ectopic pregnancies can cause serious morbidity, and the patient can die depending on her condition. Currently, methotrexate or surgical treatment is selected as treatment for

ectopic pregnancies. In many cases, surgical treatment is performed to remove the fetus and placenta from the implantation site. In addition, surgical treatment is performed in cases where methotrexate treatment is ineffective. Combination therapy with gefitinib, an epidermal growth factor receptor tyrosine kinase inhibitor, and methotrexate was suggested to be more effective than methotrexate alone. However, a recent clinical trial did not show the greater efficacy of combination therapy than methotrexate alone [5]. In addition, higher incidences of diarrhea and rash were observed with combination therapy than methotrexate alone [5].

## Conclusion

Laparoscopic surgery is performed as surgical treatment for many ectopic pregnancies worldwide. Recently, laparoscopic surgery has also been safely performed for ectopic pregnancies at rare sites. Emergency surgery has also been performed quickly and safely for cases of ectopic pregnancy with massive bleeding. Laparoscopic surgery is considered to be useful even for patients with unstable hemodynamics if there is sufficient cooperation with a general managing physician and the medical system of the facility is well established.

## Notes

- **Note 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 2000-4000 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. In most cases, the blood hCG level (discriminatory zone) is approximately 1000-2000 IU/L when the GS becomes detectable on ultrasound.
- **Note 2:** Transvaginal ultrasound imaging. An intrauterine GS image is detected at the late 4th week of gestation by transvaginal ultrasound imaging, and GS is detected in nearly 100% of cases at the early 5th week of gestation. If an intrauterine GS image is observed, ectopic pregnancies are ruled out, with the exception of simultaneous intrauterine and ectopic pregnancies, which are thought to occur at a frequency of 1/30,000. However, in cases of pregnancies via in vitro fertilization, the frequency of simultaneous internal and external pregnancies increases to 1%–3%.
- **Note 3:** In the early stages of organogenesis, male and female fetuses have two reproductive ducts (i.e., the Wolffian and Müllerian ducts). The Müllerian ducts develop outside the gonads, whereas the Wolffian ducts develop via invagination of the coelomic epithelium. Cranially, the Müllerian duct opens into a body cavity in a funnel-like fashion, and caudally, the Müllerian duct meets the opposite Müllerian duct. In the male fetus, testosterone produced by the Leydig cells of the testis stimulates the development of the Wolffian ducts. The Müllerian duct inhibitory substances made by Sertoli cells inhibit the development of the Müllerian ducts (tissues of the female reproductive tract). The Wolffian ducts remain in the fetus to form the main male reproductive tract. In the female fetus, the head of the Müllerian tube forms with the fallopian tube tissue, and the left and right Müllerian tubes are closely fused downward.

This fused portion forms the tissue of the uterine tube.

- **Note 4:** Methotrexate is a drug used to treat inflammatory diseases, such as rheumatoid arthritis and psoriasis, and malignant tumors. When methotrexate is injected in patients with ectopic pregnancies, it can terminate the pregnancy by inhibiting embryonic or fetal cell division.

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## Competing Interest statement

The authors state No competing interest.

## Ethics approval and consent to participate

This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Tokyo, Japan) on November 08, 2019, and Kyoto University School of Medicine (Kyoto, Japan) on August 17, 2019, with approval codes NHO H31-02 and M192. The completion numbers for the authors are AP0000151756, AP0000151757, AP0000151769, and AP000351128. As this research was considered clinical research, consent to participate was required. After briefing regarding the clinical study and approval of the research contents, the participants signed an informed consent form.

**Clinical Research:** A multi-center retrospective observational clinical study of subjects who underwent cancer genomic medicine at a cancer medical facility in Kyoto, Japan.

This study was reviewed and approved by the Central Ethics Review Board of the National Hospital Organization Headquarters in Japan (Tokyo, Japan) on November 18, 2020, and Kyoto University School of Medicine (Kyoto, Japan) on August 24, 2022, with approval codes NHO R4-04 and M237.

All participants agreed to take part in the present study. We have obtained Informed Consent Statements from people

participating in clinical studies.

## Author contributions

All authors had full access to the data in the study and take responsibility for the integrity of the data and accuracy of the data analysis. Conceptualization, T.H. and I.K.; Writing-Original Draft, T.H. and I.K.; Writing-Review & Editing, I.K.; Visualization, T.H. and I.K.; Supervision, T.H. and I.K.; Funding Acquisition, T.H. and I.K.

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