**Successful *in vitro* fertilization pregnancy and delivery in an infertile patient with
ovotesticular disorder of sex development: a case report**

Running title: *In vitro* fertilization pregnancy in DSD

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Abstract

A 29-year-old nulliparous woman was diagnosed with ovotesticular disorder of sex development (DSD) based on postoperative histopathological findings after undergoing unilateral gonadectomy at the age of 6 years; later (age of 8 years), she had also undergone vulvoplasty and vaginoplasty. Her karyotype was 46, XX. She had dyspareunia because of a narrow vagina, but had a normal uterus and left gonad. Spontaneous ovulation was confirmed, but sexual intercourse was impossible because of dyspareunia despite performing vaginal self-dilatation using a vaginal dilator. Artificial insemination was initiated; however, five cycles failed to yield a viable pregnancy. We decided to perform *in vitro* fertilization (IVF), which resulted in conception. To reduce her distress during IVF because of insufficient lumen expansion following vaginoplasty, we administered adequate intravenous anesthesia before oocyte collection. The patient delivered a healthy male infant weighing 2,558 g at 37 weeks of gestation via cesarean section, which was performed because of gestational hypertension. This is the eighth report of a viable neonate born from a patient with ovotesticular DSD following gonadectomy and the first such pregnancy achieved by IVF. Therefore, IVF seems to be an effective option for infertile patients with ovotesticular DSD. Additionally, self-management of the plastic vagina is important during the peri- and postoperative periods of early vaginoplasty to prevent dyspareunia.

Keywords: dyspareunia, gonadectomy, *in vitro* fertilization, ovotesticular, pregnancy

Introduction

Ovotesticular disorder of sex development (DSD) is a condition of incomplete sexual differentiation, characterized by the presence of testicular and ovarian tissues in the same individual. Although rare, pregnancies have been reported in patients who had and had not undergone unilateral gonadectomy for ovotesticular DSD¹. However, to the best of our knowledge, only cases of natural pregnancies in patients with ovotesticular DSD who had undergone unilateral gonadectomy have been reported in the literature to date.

Here, we present a successful case of assisted pregnancy and delivery achieved by *in vitro* fertilization (IVF) in an infertile patient with ovotesticular DSD who had previously undergone unilateral gonadectomy.

Case Report

History

The patient was a 29-year-old nulligravid and nulliparous woman who initially presented to a specialist with a chief complaint of dyspareunia. She was married and desirous of children and was subsequently referred to our hospital for detailed clinical examination and treatment.

An abnormal vulval morphology had been noted at her birth. At the age of 6 years, prior to the surgery, an enlarged clitoris and no vagina were observed. A swelling, which was diagnosed as ovotestis postoperatively, was palpable at her right groin. Thereafter, she underwent unilateral gonadectomy at another hospital, and the diagnosis of ovotesticular DSD was confirmed histopathologically. At the age of 8 years, she underwent reconstructive surgery (vulvoplasty and vaginoplasty). Unfortunately, the surgical records were unavailable; therefore, further details could not be ascertained.

64

65 *Physical examination (at first visit)*

66 The patient's height and weight were 157 cm and 52 kg, respectively, and the external
67 features (breasts and pubic hair) appeared feminine. She had female genitalia, with
68 distinct labia majora, labia minora, and a clitoris; the constructed vagina was located
69 anterior to the labia minora, which were fused. On pelvic examination, the patient's
70 vagina was narrow, allowing for the insertion of only one finger. Her uterus was normal
71 in size and anteverted.

72

73 *Investigations*

74 Transrectal ultrasonography revealed an 83-mm uterus in length with a 4.5-mm
75 endometrial thickness. In addition, an enlarged area because of adenomyosis (35 mm)
76 was observed in the uterus. The left gonad was 31 mm in size, and the antral follicles
77 were visible; the right gonad could not be identified (Fig. 1). Furthermore, we suggested
78 performing hysterosalpingography; however, she denied consent out of fear.

79 The patient's serum hormone levels during menstruation were as follows:
80 luteinizing hormone, 3.8 mIU/mL; follicle-stimulating hormone, 7.7 mIU/mL; estradiol
81 (E₂), 29.0 pg/mL; anti-Müllerian hormone (AMH), 1.98 ng/mL; total testosterone, 0.35
82 ng/mL; thyroid-stimulating hormone, 1.94 µIU/mL; free triiodothyronine, 3.1 pg/mL;
83 free thyroxine, 1.5 ng/mL; and prolactin, 10.5 ng/mL. In addition, she tested negative
84 for immunoglobulin (Ig)A and IgG antibodies against *Chlamydia trachomatis*. Her
85 karyotype was 46, XX.

86 Her partner's semen analysis showed a semen volume of 1.6 mL, sperm count
87 of 107 million/mL, and sperm motility of 72%.

88

89 *Treatment and course*

90 We proceeded to expand the vagina using a vaginal dilator. The patient began to
91 self-dilate once it reached a diameter of 20 mm, achieving further expansion to 30 mm
92 in 6 months. While spontaneous ovulation was confirmed, sexual intercourse was
93 impossible because of the associated pain. Concerning dyspareunia, artificial
94 insemination was initiated; however, five cycles failed to yield a viable pregnancy. Thus,
95 for this case of infertility, because of an unknown cause, we decided to perform IVF.

96 Two cycles of oocyte retrieval procedure were performed. The
97 gonadotropin-releasing hormone antagonist protocol (0.25 mg daily when the leading
98 follicle is 14–15 mm) was used in both IVF cycles. The E2 and progesterone (P4) levels
99 of each cycle were 2,606 pg/mL and 0.56 ng/mL and 2,731 pg/mL and 0.05 ng/mL,
100 respectively, on the day of human chorionic gonadotropin (hCG) administration. Both
101 oocyte retrieval procedures were performed transvaginally at 36 h after hCG injection.
102 Oocytes were harvested under intravenous anesthesia with transvaginal ultrasound
103 guidance. Eight and nine oocytes were retrieved in each cycle. The retrieved oocytes
104 were prepared for conventional IVF. The two cycles of oocyte retrieval procedure
105 yielded six frozen blastocysts in total.

106 We conducted various tests before embryo transfer (ET). Hysteroscopy
107 revealed some shrinkage of the uterine cavity (lumen); the oviducts were visible
108 bilaterally (Fig. 2a). No findings indicative of chronic endometritis were observed. Then,
109 the biopsied endometrial tissue was stained using cluster of differentiation
110 138-immunohistochemistry and viewed under high-magnification microscopy. One or
111 more plasma cells were observed in four out of 10 fields in total, leading to a diagnosis

of chronic endometritis. We administered doxycycline hyclate (Vibramycin®; Pfizer Inc., New York, NY, USA). In addition, uterine adenomyosis was confirmed using contrast-enhanced pelvic magnetic resonance imaging (MRI) (Fig. 2b).

A frozen-thawed single embryo was transferred 5 days after the initial P4 application. Embryo transfers were performed under real-time transvaginal and transabdominal ultrasound guidance, using KITAZATO ET Catheter® (Kitazato Corp., Tokyo, Japan). She conceived after the second frozen-thawed ET.

A pregnancy-related follow-up examination was performed at Nippon Medical School Hospital. An elective cesarean section had been initially scheduled for week 38 because of her past history of reconstructive surgeries. The patient's pregnancy course was unremarkable until week 36, when she developed gestational hypertension without any signs of pre-eclampsia, such as proteinuria or severe hypertension; she delivered the infant via cesarean section at week 37 (male, 2,558 g), 1 week earlier than scheduled because of concerns regarding gestational hypertension worsening. Her systolic and diastolic blood pressures prior to delivery were <160 and <100 mmHg, respectively, and became normal after 1 day. Intraoperative findings showed that the uterus was extensively adhered to the peritoneum, and her left gonad was detected as an ovary of regular size. The infant's Apgar scores at 1 and 5 min after the delivery were 7 and 9, respectively. He needed respiratory care on the 1st day at the Growing Care Unit, where he left a day after.

Discussion

We describe our treatment methodology in the case of an infertile woman with

ovotesticular DSD, who had undergone unilateral gonadectomy in her childhood, with IVF resulting in conception and live birth. To date, there are limited reports on pregnancies in such cases; this is only the eighth successful delivery documented among women with this condition post-gonadectomy. The seven previous cases had resulted from natural conception, making this the first IVF pregnancy and viable delivery reported in a patient with ovotesticular DSD post-gonadectomy in the literature.

Successful birth from a woman with ovotesticular DSD following gonadectomy is a rare phenomenon. Zeki et al. reported 26 pregnancy cases among women with ovotesticular DSD¹; specifically, they reported seven and 19 cases of pregnancy in patients with ovotesticular DSD who had and had not undergone gonadectomy, respectively (Table 1). Only one of the seven women had undergone vaginoplasty at the age of 15 years while continuing dilation therapy; intercourse was initially painful after colporrhaphy, and pelvic inflammation occurred because of intercourse⁵. However, she conceived naturally at the age of 22 years, and her child was delivered by cesarean section.

According to the literature published to date, including the aforementioned report by Zeki et al., ovotesticular DSD does not appear to be involved in gestational hypertension. As our patient did not have a history of hypertension and/or any specific risk factors, such as obesity or family history, the development of gestational hypertension was considered accidental.

In all reported cases, including the present case, the neonate was male. There have been two theories regarding the fact that all deliveries of ovotesticular DSD were male infants¹. First, the functional ovary in ovotesticular DSD is usually located on the left side⁸. The venous drainage of the ovaries makes the left ovary warmer than the right

ovary, which may favor a sperm with a Y chromosome. The second theory is related to the possible characteristics of the maternal embryo's disorders in fertilization and embryonic development⁷. Embryos formed by oocytes fertilized by sperm carrying an X chromosome may not survive because of a genetic disorder involving the sex chromosomes or autosomes. For example, an abnormal maternal X chromosome with Y genes may not pair well with a normal paternal X chromosome at fertilization. Possibly, pairing of the abnormal X with the shorter Y chromosome during early embryogenesis may not be a problem in this rare condition. As this was the first such case where IVF was performed, the detailed results would support these theories. In the present case, there was no obvious abnormality in fertilization or embryonic development; nevertheless, our findings were insufficient to completely rule out the latter theory, mainly because preimplantation genetic testing was not performed. There are four last frozen blastocysts, and the patient has a desire to have more children. Although there is a risk of performing repeated cesarean sections, it is important to confirm the sex of the subsequent children or examine the chromosomes of the miscarried tissue if the pregnancy ends in abortion.

In cases of vaginal hypoplasia, reconstructive surgery is one option for ovotesticular DSD, although practitioners should also consider self-management in the peri- and postoperative periods. Vulval and vaginal morphologies are diverse among ovotesticular DSD; while no precise taxonomy has been established, three types are usually considered: masculine, feminine, and "ambiguous." Takezaki et al. reported that most patients with ovotesticular DSD with one ovary and one ovotestis have a feminine vulva and are raised as girls⁹. Similarly, our patient was born with one ovary and one ovotestis and was raised as a girl. An interview with the patient revealed that she was

184 diagnosed with ovotesticular DSD at the age of 6 years after undergoing unilateral
185 gonadectomy, based on the postoperative histopathological findings. However, surgical
186 and pathological records were unavailable, and further details could not be obtained.
187 She also underwent vulvoplasty and vaginoplasty at the age of 8 years. Despite our
188 efforts to dilate the vaginal cavity 20 years after this reconstructive surgery, the
189 persistence of painful intercourse necessitated a strategic shift to artificial insemination.
190 Opinions differ concerning the optimal timing of vaginoplasty. Especially, Jong et al.
191 recommended that it should be performed during the neonatal period when the internal
192 and external genitalia are relatively well developed because of the uterus's high
193 estrogen environment¹⁰. Specifically, the timing should be based on the patient's
194 compliance with self-management postoperatively and the presence of a willing sexual
195 partner in the present or near future. Mekaru et al. performed vaginal reconstruction on
196 a 26-year-old woman with Mayer–Rokitansky–Küster–Hauser syndrome but reported
197 that her vaginal cavity shrank after the procedure because of negligent self-management
198 during periods without a sexual partner¹¹.

199 The outcome in our case was a successful IVF pregnancy and relatively early
200 delivery. This outcome could be a harbinger of good news for infertile women with
201 ovotesticular DSD. Typically, IVF is not considered until women reach the age of
202 30 years; however, our patient's AMH titer was approximately half the standard for
203 women of her age, although it was comparable to the expected value for a woman of her
204 age with only one functional ovary, suggesting a healthy ovarian reserve¹². Indeed, her
205 course was favorable for a woman of her age, leading to pregnancy 9 months after the
206 first IVF procedure, indicating that unilateral gonadectomy is unlikely to significantly
207 affect IVF outcomes among patients with ovotesticular DSD.

Practitioners should be aware that insufficient lumen expansion following vaginoplasty complicates oocyte collection and ET. Technical difficulties have been reported during oocyte retrieval due to a short, inelastic neovagina^{13,14}. Our patient reported that pain and fear were associated with transvaginal procedures; thus, we administered adequate intravenous anesthesia before oocyte collection to reduce the distress. In addition, contrast-enhanced pelvic MRI and hysteroscopy were performed before ET. The latter was successfully performed with combined transvaginal and transabdominal ultrasonography.

In conclusion, this is the first case report of a successful IVF pregnancy and viable delivery in an infertile patient with ovotesticular DSD who had previously undergone unilateral gonadectomy. Early vaginoplasty in patients with ovotesticular DSD may be ineffective in the reproductive age because the vaginal cavity needs to be dilated to prevent dyspareunia. IVF is an effective option for infertile patients with ovotesticular DSD to achieve successful conception and birth.

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225

226 **Conflict of interest**

227 The authors have no conflicts of interest to declare.

228

229 **Informed consent**

230 The patient provided informed consent to use the clinical results for our research.

231

232 **Human/animal rights:** This article does not contain any studies with human and
233 animal subjects performed by any of the authors.

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270 **Figure legends**

271 **Fig. 1.** Images of transrectal ultrasonography

272 Left: Uterus with adenomyosis. Right: Left ovary with the antral follicles visible; the
273 right ovary could not be identified.

274

275 **Fig. 2.** Images of the tests before embryo transfer

276 A) Hysteroscopic image of the uterine cavity. Left: Uterine cavity (lumen). Center:
277 Right oviduct. Right: Left oviduct. B) An enhanced magnetic resonance image with
278 T2-weighted imaging of the pelvis showing the uterus with adenomyosis.

279

Figure 1.

Figure 2a.

Figure 2b.

1 Table 1. Pregnancies and births in cases of ovotesticular disorder of sex development
 2 after gonadectomy

Studies	Karyotype	Age (years) at gonadectomy	Age (years) at vulvoplasty	Age (years) at vaginoplasty	Age (years) at pregnancy	Delivery	Infant
Narita et al., 1975 ²	46, XX	25	25	None	34	Cesarean section	Male
Williamson et al., 1981 ³	46, XX	6	6	None	18	Vaginal	Male
Minowada et al., 1984 ⁴	46, XX	14	4	None	24	Vaginal	Male
Starceski et al., 1988 ⁵	46, XX	0	1	15	23	Cesarean section	Male
Pereira et al., 1991 ⁶	46, XX	13	None	None	Unknown	Vaginal	Male
	46, XX	13	None	None	Unknown	Vaginal	Male
Schultz et al., 2009 ⁷	46, XX	0	0	None	21	Vaginal	Male
Present Case	46, XX	6	8	8	30	Cesarean section	Male