Female pseudohermaphroditism associated with maternal steroid cell tumor, not otherwise specified of the ovary: a case report and literature review

K. Hasegawa[1], Y. Minami[1], H. Inuzuka[1], S. Oe[1], R. Kato[1], K. Tsukada[1], Y. Udagawa[1], M. Kuroda[2]

[1]Department of Obstetrics and Gynecology, and [2]Department of Pathology, Fujita Health University School of Medicine, Toyoake, Aichi (Japan)

Summary

Maternal virilization in pregnancy with or without fetal female pseudohermaphroditism has several etiologies. Of these, pregnancy luteoma is the most common cause of maternal virilization during pregnancy, and approximately 20 cases have been reported in recent years. Moreover, four cases of pregnancy luteomas with female pseudohermaphroditism have been reported. However, the extremely rare steroid cell tumor, not otherwise specified (NOS), has been reported only once as a cause for maternal virilization. Herein, the authors report the first case of maternal virilization with female pseudohermaphroditism associated with steroid cell tumor-NOS along with the clinical course, pathological features, and a review of the literature.

Key words: Female pseudohermaphroditism; Maternal virilization; Steroid cell tumor; Not otherwise specified; testosterone; Pathological diagnosis.

Introduction

Steroid cell tumors of the ovary are rare and account for approximately 0.1% of all ovarian tumors. These are classified into three subtypes: stromal luteomas, Leydig cell tumors, and steroid cell tumors, not otherwise specified (NOS), which account for approximately 60% of all steroid cell tumors [1]. Steroid cell tumors-NOS produce virilization in 56%-77%, hyperestrogenism in 6%-23%, and Cushing's syndrome in 6%-10% of cases [2, 3]. Steroid cell tumors-NOS may produce the full range of steroid hormones seen in the other types. As menstrual abnormalities are common, pregnancies in the setting of this tumor are very rare. Only one case of maternal virilization by a steroid tumor NOS in pregnancy with a male fetus has been reported in the literature [4], and this case was not associated with fetal female pseudohermaphroditism. Herein, the authors report a case of maternal virilization and female pseudohermaphroditism caused by steroid cell tumor-NOS, along with the clinical course, histopathological features, and the literature review.

Case Report

A 36-year-old primigravida woman was admitted to this hospital with preterm rupture of membranes and the onset of labor at 22 weeks of gestation. She had a history of an exploratory laparotomy five years prior for bilateral solid ovarian tumors, which were initially suspected to be malignant. A left salpingo-oophorectomy was performed, and the tumor was thought to be benign on intraoperative gross inspection. The right ovarian tumor was not removed so as to not compromise fertility. The left ovarian tumor was initially diagnosed as a leiomyoma. The original tissue blocks were not available at the time this case report was drafted. The patient had irregular menstrual cycles since menarche at age 11. She was treated for infertility for four years and eventually became pregnant following ICSI (intracytoplasmic sperm injection). Magnetic resonance imaging (MRI) performed at 15 weeks of gestation, prior to referral showed a 75 x 80 mm solid tumor in the right pelvis, with heterogeneous low to intermediate signal intensity on T2-weighted imaging without contrast enhancement (Figure 1a).

On admission, her height was 160 cm, weight 51 kg, and her blood pressure was 118/70 mmHg. She presented with virilization manifested by increased facial, abdominal, and lower extremity hair, worsening acne, and a slightly enlarged clitoris. Virilization of the patient was not noticed in previous hospital during treatment for infertility. On ultrasonography, a 76 x 71 x 80 mm solid tumor was detected in the pouch of Douglas; Doppler evaluation of the tumor demonstrated hypovascularity. There was no morphological abnormality in the maternal adrenal gland. The fetus measured appropriate for gestational age and had normal anatomy and appeared to have male genitalia; however, the structure of the scrotum was obscure. The levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), thyroid-stimulating hormone (TSH), and free-T3 or T4 were all within the normal range. The serum testosterone level was markedly elevated (32 ng/ml, normal range; 0.1~0.7 ng/ml). The levels of serum tumor markers, carcinoembryonic antigen (CEA), CA19-9 were normal; however, the CA125 was slightly elevated (73 U/ml). A right ovarian sex-cord stromal tumor that produced testosterone was suspected, based on the physical, laboratory, and radiological findings. The patient was treated for preterm labor after admission to this hospital; however, she eventually developed chorioamnionitis and entered into active labor at 29 weeks of gestation. She delivered by cesarean section and underwent a right ovarian cystectomy at the same time. There was no evidence of extra-ovarian tumor or metastatic disease. A small amount of ascites was seen in the cul de sac.

The tumor measured seven by eight cm in diameter and was a well-circumscribed, grayish-yellow mass without apparent area of necrosis or degeneration (Figure 1b). The tumor was easily separated from the grossly normal-appearing ovarian tissue. Cytological examination of ascites was negative for malignancy. Histological examination of the tumor demonstrated an encapsulated, non-infiltrative pattern. The tumor included areas in which cuboidal or polygonal cells with oval to polygonal nuclei, small dis-

Revised manuscript accepted for publication November 19, 2012

Table 1. — *Cases of female pseudohermaphroditism.*

Authors	Mat. age	Gravida and para	Gestat. weeks at diagnosis	Tumor size at diagnosis (mm)	Radiological findings	Histological or clinical diagnosis	Maternal testosterone level	Prader class	Maternal therapy	Maternal outcome	Fetal outcome
Massa V. [8]	34	nulligravida	13	R: 50	US; small hypo-echoic and hyperechoic area	bilateral pregnancy luteoma	T: 2,000 ng/ml (normal range: 50-300 ng/ml) ADS: 6,500 ng/ml (normal range: 100-250 ng/ml)	V	cesarean section with bil-tumorectomy at 39 weeks gestation	improvement of hirsutism	feminizing genitoplasty
Wang Y.C. [9]	27	nulligravida	35	R: 70x60x50 L: 90x64x50	MRI; solid mass and multiple nodules	bilateral pregnancy luteoma	T: 11,539 ng/ml (normal range: 20-86 ng/ml)	NS (I?)	vaginal delivery at 36 weeks	bilateral ovaries normalized and improvement of hirsutism	NS
Spitzer R.F. [6]	36	nulligravida	post-partum	R: 73x74x47	MRI; heterogeneously; predominantly solid	r-pregnancy luteoma	T: 10.6 nmol/l (normal range: < 2.9 ng/ml)	II-III	vaginal delivery at 36 weeks and RSO and OMT on the 18th postpartum	improvement of hirsutism	considering urogenital sinus repair
Ugaki H. [10]	33	nulligravida	35	L: 60	NS	l-pregnancy luteoma	T: 6.11 ng/ml (normal range: 0.85 ± 0.28 ng/ml)	NS (III?)	cesarean section with LSO at 35 weeks gestation	improvement of hirsutism	feminizing genitoplasty
Current case	36	nulligravida	15	R: 75x80	heterogeneous low to interme-diate signal inten-sity on T2-wighted imaging	r-SCT-NOS	T: 32 ng/ml (normal range: 0.1-0.7 ng/ml)	V	cesarean section with right-ovarian tumorectomy at 29 weeks gestation	improvement of hirsutism	died of cerebellar tumor

R: right, L: left, T: Testosterone, ADS: Androstenedione, NS: not specified, RSO: right salpingo-oophorectomy, OMT: omentectomy, LSO: left salpingo-oophorectomy.

tinct nucleoli, and abundant eosinophilic cytoplasm were arranged in a diffuse pattern of columns or nests. These columns were surrounded by spindle cells with central, small, round-to-oval nuclei with small nucleoli. These cells lacked typical Reinke's crystals commonly seen in Leydig cell tumors. Only a few microscopic areas of necrosis were identified. The cellular atypia was scant and mitotic figures were found in less than two per ten high-power fields (Figure 2a, 2b). Both cell types were focally positive for fat stains by oil red and Sudan III.

Immunohistochemical staining was performed for AE1/AE3 (anion exchange protein) (1: 100 dilution), CAM 5.2 (1: 40 dilution), alpha-smooth muscle actin (SMA) (clone 1A4, 1: 200 dilution), vimentin (clone V9, 1: 400 dilution), desmin (clone DE-R-11, 1: 200 dilution), inhibin-alpha (clone R1, 1: 50 dilution), estrogen receptor (clone SP1, 1: 2 dilution), progesterone receptor (clone 1E2, 1: 2 dilution), testosterone (1: 50 dilution), and Ki-67 (clone MIB-1, 1:100 dilution) using the streptavidin-biotin-peroxidase complex method. Consequently, immunohistochemical staining of the two-component cell types was negative for cytokeratin (CAM5.2 and AE1/AE3), estrogen receptor, and progesterone receptor, and positive for inhibin-alpha and vimentin. Only the spindle cells were positive for smooth muscle actin SMA and desmin. Importantly, testosterone staining was positive in both components (Figure 2c~2f). The Ki-67 labelling index was 2.5% throughout the specimen. The final pathological diagnosis was a steroid cell tumor-NOS of the ovary.

The maternal serum testosterone level immediately normalized following tumor resection and her hirsutism slowly decreased. She has had no evidence of recurrence for five years, and her serum testosterone level has remained normal.

The neonate weighed 1,280 g at birth, and had Apgar scores of 9 at one minute and 9 at five minutes. The neonate was admitted to the neonatal intensive care unit. The neonate had ambiguous genitalia with a small penis without an obvious scrotum or palpable testis in the inguinal or genital region. Cytogenetic investigation on blood lymphocytes of the baby revealed a normal female karyotype 46, XX and was negative for the SRY gene. The neonate exhibited complete masculinization of the external genitalia with the external urethral meatus opening at the apex of the penis and complete labial fusion (Prader type V) [5] (Figure 3a). MRI findings revealed a small uterus; the uterine corpus and the cervix were not distinguishable, and the vagina was closed just beneath the labia (Figure 3b). While the neonate initially grew normally, she developed hydrocephalus secondary to a cerebellar tumor. The tumor was resected and was consistent with a medulloblastoma with extensive nodularity and advanced neuronal differentiation. The tumor recurred and the infant died of disease progression at one year of age.

Discussion

The differential diagnosis of maternal virilization in pregnancy is divided into adrenal, ovarian, and iatrogenic causes. Ovarian tumors or tumor-like lesions which produce androgens include pregnancy luteoma, hyperreactio luteinalis, granulosa cell tumor, thecoma, Sertoli-Leydig cell tumor, steroid cell tumors including pure Leydig cell tumor, stromal luteoma and steroid cell tumor-NOS, stromal hyperthecosis, and ovarian tumors with functioning stroma including cystadenoma, cystadenocarcinoma, Brenner tumor, dermoid cyst, and Krukenberg (metastatic) tumor [6].

Pregnancy luteoma is the most common cause of maternal virilization during pregnancy, and approximately 20 cases have been reported in recent years [7]. Moreover, four cases of pregnancy luteomas with female pseudohermaphroditism have been reported in the English literature [6, 8-10]. This lesion is characterized by spontaneous disappearance of the tumors and normalization of the androgen levels after delivery. Imprudent surgical intervention should be withheld except for ovarian torsion or obstructed labour. However, three of four cases with female pseudohermaphroditism underwent surgery to obtain the accurate pathological findings at the same time of cesarean section or puerperium. Wang *et al.* reported a case of a nulligravida woman suffering from bilateral hydronephrosis and recurrent pyelonephritis caused by

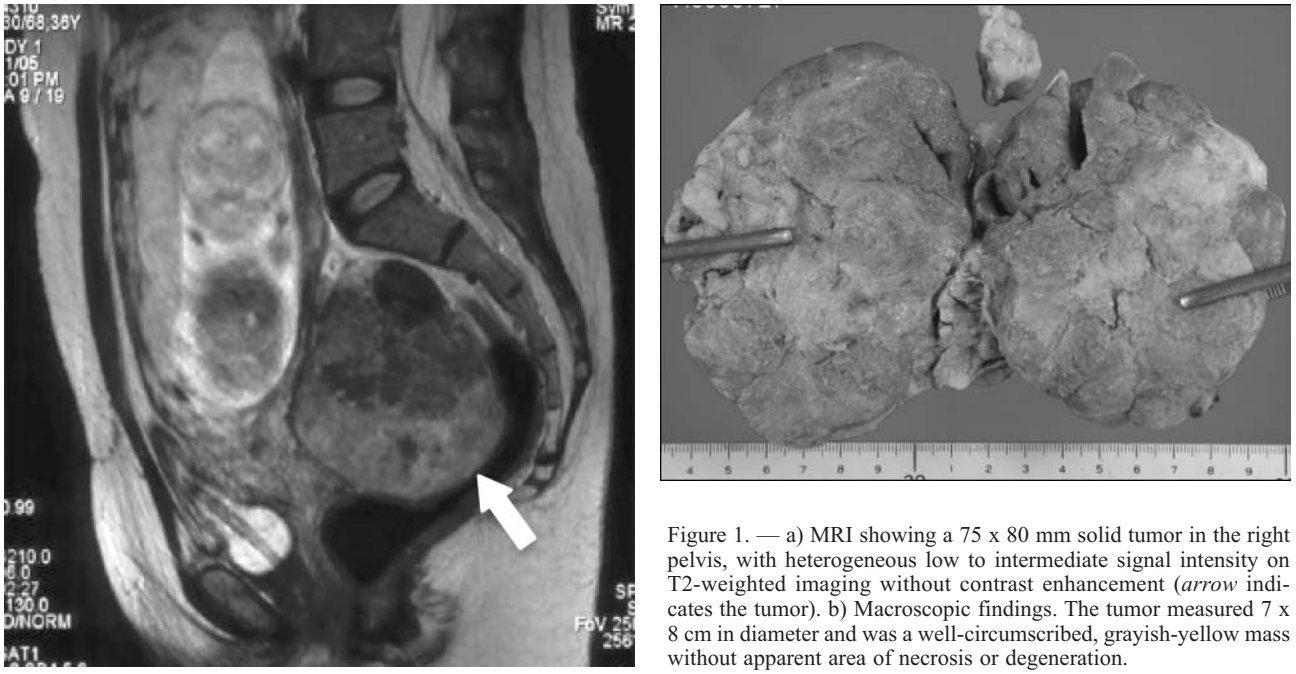

Figure 1. — a) MRI showing a 75 x 80 mm solid tumor in the right pelvis, with heterogeneous low to intermediate signal intensity on T2-weighted imaging without contrast enhancement (*arrow* indicates the tumor). b) Macroscopic findings. The tumor measured 7 x 8 cm in diameter and was a well-circumscribed, grayish-yellow mass without apparent area of necrosis or degeneration.

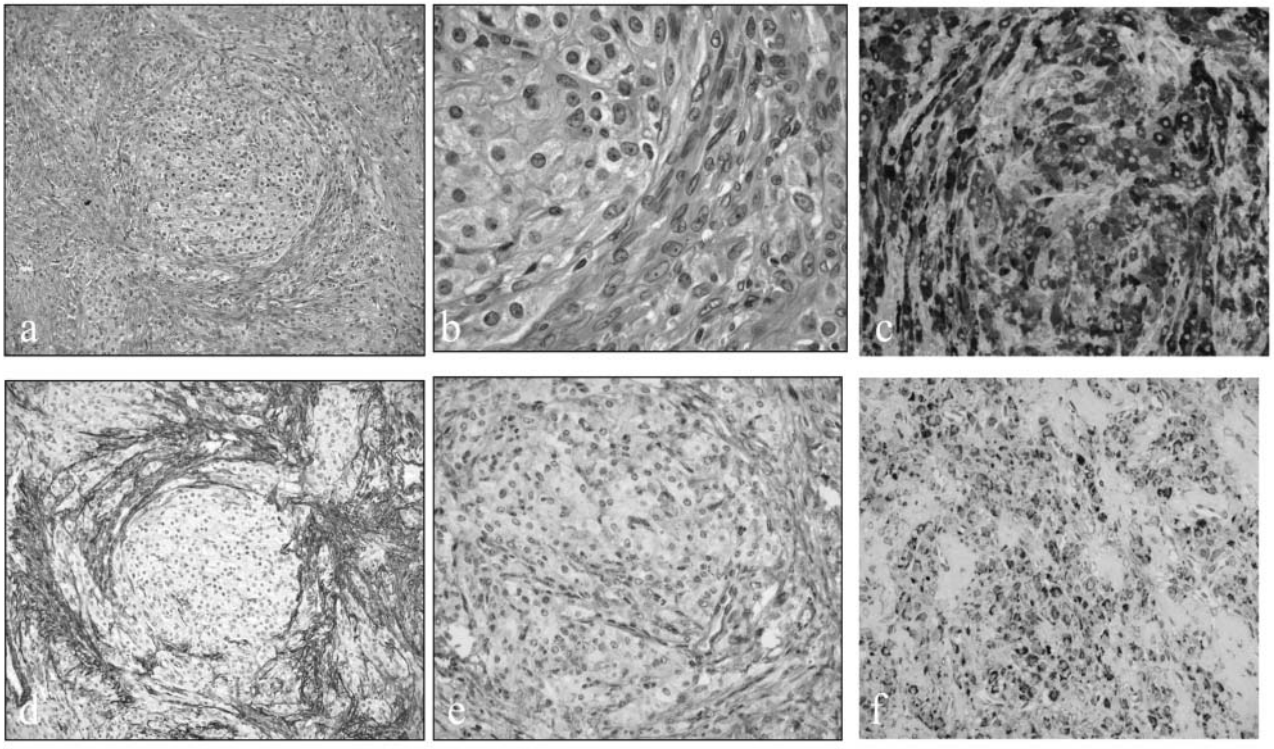

Figure 2. — a), b) Pathological examination. The tumor includes areas in which cuboidal or polygonal cells with oval to polygonal nuclei, small distinct nucleoli, and abundant eosinophilic cytoplasm are arranged in a diffuse pattern of columns or nests. These columns are surrounded by spindle cells with central, small, round-to-oval nuclei with small nucleoli. These cells lack typical Reinke's crystals. Only a few microscopic areas of necrosis are identified. The cellular atypia are scant and mitotic figures are found in less than two per ten high-power fields. [a) hematoxylin and eosin (H&E), original magnification x100, b) H&E, x400] c), d), e), and f) Immunohistochemical study. c) inhibin-alpha (original magnification x 200), d) SMA (x100), e) vimentin (x200), f) testosterone (x 200).
The two-component cell types are positive for inhibin-alpha, vimentin, and testosterone. Only the spindle cells are positive for SMA.

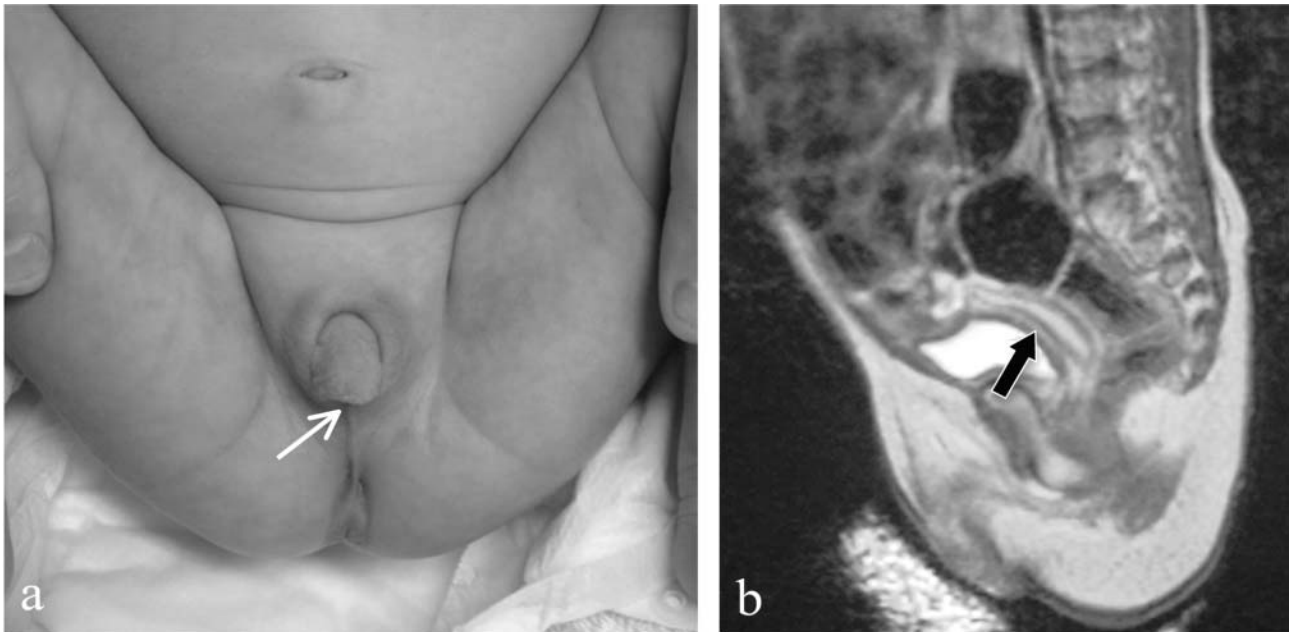

Figure 3. — a) External genitalia of the neonate. The neonate exhibited complete masculinization of the external genitalia with the external urethral meatus opening at the apex of the penis and complete labial fusion (Prader type V). (arrow indicates the external urethral meatus). b) MRI findings revealing a small uterus; the uterine corpus and the cervix are not distinguishable, and the vagina is closed just beneath the labia (arrow indicates the uterus).

bilateral solid ovarian tumors presented maternal virilization [9]. The unique MRI imaging features of this case were reported by Kao *et al*. as follows; intermediate high signal and contrast enhanced on T1, and low signal on T2-weighed images [11]. Based on the clinical and MRI imaging, bilateral ovarian tumors of this case were diagnosed as pregnancy luteoma, and this case was subsequently avoided from surgical intervention at pre- or post-partum [9, 11]. Moreover, this woman conceived her second pregnancy with a female fetus of 46, XX karyotype. Her pregnancy was terminated at 14 weeks gestation because of suffering from pregnancy luteoma repeatedly (maternal serous testosterone level; 751 ng/ml), and fetal ambiguous external genitalia with clitoral hypertrophy was confirmed [12]. She conceived a third pregnancy with a male by Y-bearing spermatozoa for intrauterine insemination as a male preselection. A healthy boy was born without disorder of sex development at 35 weeks gestation by cesarean section with bilateral pregnancy luteoma enlarged up to ten cm in diameter and elevated serum testosterone; 12,400 ng/ml [12].

However, the almost androgen-producing ovarian tumors, except for pregnancy luteoma, do not regress spontaneously after delivery, so the differential diagnosis of an ovarian tumor during pregnancy is important and essential for further management.

There has, however, been only one report of maternal virilization caused by a steroid cell tumor-NOS during pregnancy [4]. Vulink *et al*. reported a 37-year-old pregnant woman who showed progressive hair growth on her face, arms, and legs, deepening of the voice, and slight enlargement of the clitoris. A solid, homogenous tumor of the left ovary was detected by ultrasonography at 12 weeks of gestation along with elevated serum testosterone. She underwent an exploratory laparotomy with left salpingo-oophorectomy at 16 weeks of gestation, and the histopathological findings were consistent with a benign ovarian steroid cell tumor-NOS. As the fetus was male, there were no visible effects of testosterone exposure. To the best of the authors' knowledge, the present case is the first to report female pseudohermaphroditism associated with maternal steroid cell tumor-NOS of ovary.

Differentiation of the female external genitalia occurs between the seventh and 12th week of gestation. Increased exposure to androgens during this critical period results in labial fusion. After the 12th week of gestation, labia and clitoral hypertrophy may be induced [13, 14]. Almost all previously reported cases of female pseudohermaphroditism caused by pregnancy luteoma have been Prader type I to III (Table 1) [7]. However, Mazza *et al.* reported a case with Prader type V fetal masculinization [6]. They identified the duration and timing of embryo-fetal androgen exposure, a deficit of protective factors, and fetal organ sensitivity as influencing the degree of fetal masculinization. The duration of embryo-fetal androgen exposure in the present case with maternal steroid cell tumors-NOS was longer than in those of cases with a pregnancy luteoma and likely explains the complete female masculinization.

Steroid cell tumors typically are solid and well-circumscribed and are rarely lobulated [15]. These tumors are bilateral in six percent of cases [2]. Steroid cell tumors-NOS occur at any age with average age of diagnosis of 43 years [2]. These tumors are larger than the other steroid cell tumors; with cases ranging from 1.2 to 45 cm in diameter [2, 16]. Histopathologically, steroid cell tumors-NOS can be

differentiated from stromal luteomas, which are confined within ovarian stroma and commonly associated with stromal hyperthecosis. They are also distinguishable from Leydig cell tumors which contain cytoplasmic Reinke crystals [1]. Steroid cell tumors-NOS are composed of two types of cells: cells with abundant eosinophilic, slightly granular cytoplasm, and cells with vacuolated cytoplasm [2]. These cells are most commonly arranged in a diffuse pattern but are occasionally seen in nests and columns. The stroma is sparse, consisting of delicate connective tissue supporting a rich vascular network, and is occasionally fibrous or hyalinized [2]. These tumors are commonly positive for inhibin-alpha and vimentin, and negative for cytokeratin. They have recently been shown to be positive for calretinin and Melan A [17, 18]. In the present case, the histopathological findings were not typical for a steroid cell tumor-NOS. The tumor consisted of large cells with abundant pale or eosinophilic cytoplasm, as well as spindle cells. The former cells were consistent with those found in a steroid cell tumors-NOS. Inhibin-alpha and vimentin were positive and cytokeratin was negative for both cell types, whereas SMA and desmin were positive only in the spindle cells. These spindle cells were thought to be differentiating to smooth muscle cells, which may have been what prompted the diagnosis of leiomyoma for the previously resected left ovarian tumor in this patient.

The majority of steroid cell tumors-NOS are benign. Despite the majority being low-grade, approximately 25%-43% of these tumors are malignant in adults [2, 19]. In a review of 63 cases, the pathological features associated with malignant behavior are: two or more mitotic figures per ten high-power fields (92% malignant); necrosis (86% malignant); tumor diameter of more than seven cm (78% malignant); hemorrhage (77% malignant); and grade 2-3 nuclear atypia (64% malignant) [2]. The average age of patients with malignant steroid cell tumors-NOS is higher than that of patients with benign tumors, of 54 and 38 years, respectively [2]. The tumor in the present case showed benign pathological features with the exception of the tumor measuring seven by eight cm. Since the patient was treated with a cystectomy, she has been followed closely with monitoring of the serum testosterone level and has shown no evidence of recurrence.

Two of four masculinized females caused by pregnancy luteoma underwent feminizing genitoplasty, and one case was considering urogenital sinus repair at appropriate age in the literature (Table 1). As for the present case, the neonate died of her cerebellar tumor before the planning of postnatal medical care, gender assignment, and the timing of feminizing genitoplasty. The association between the cerebellar tumor and maternal testosterone excess is uncertain.

Steroid cell tumors-NOS produce the full spectrum of hormonal perturbations seen with other steroid cell tumors. Therefore, they frequently result in primary or secondary infertility. The present patient became pregnant by ICSI after a four-year history of infertility treatment. The serum testosterone level during infertility treatment of the present case was not available without the awareness of virilization in previous hospital. While the infertility was circumvented, the effects on the fetus remained. The present case also illustrates a potential pitfall of artificial reproductive technology.

References

[1] Young R.H., Scully R.E.: "Sex cord-stromal and steroid cell ovarian tumors". Kurman R.J., Blaustein's Pathology of the Female Genital Tract. 5th ed. Pp 905, Springer-Verlag, New York, 2001.

[2] Hayes M.C., Scully R.E.: "Ovarian steroid cell tumors (not otherwise specified). A clinicopathological analysis of 63 cases". *Am. J. Surg. Pathol*., 1987, *11,* 835.

[3] Reedy B., Richards W.E., Ueland F., Uy K., Lee E.Y., Bryant C., van Nagell J.R. Jr.: "Ovarian steroid cell tumours, not otherwise specified: a case report and literature review". *Gynecol. Oncol.,* 1999, *75*, 293.

[4] Vulink A.J.E., Vermes I., Kuijper P., ten Cate L.N., Schutter E.M.: "Steroid cell tumour not otherwise specified during pregnancy: a case report and diagnostic work-up for virilisation in a pregnant patient". *Eur. J. Obstet. Gyecol.*, 2004, *112,* 221.

[5] Prader A.: "Disorders of sexual differentiation (Intersexuality)". In: Labhart A. (ed.) Clinical Endocrinology Theory and Practice, Springer Verlag, 1986, 715.

[6] Spitzer R.F., Wherrett D., Chitayat D., Colgan T., Dodge J.E., Salle J.L., Allen L.: "Maternal luteoma of pregnancy presenting with virilization of the female infant". *J. Obstet. Gynecol. Can.,* 2007, *29,* 835.

[7] Masarie K., Katz V., Balderston K.: "Pregnancy luteomas- Clinical presentation and management strategies". *Obstet. Gynecol. Surv.,* 2010, *65,* 575.

[8] Mazza V., Di Monte I., Ceccerelli P.L., Rivasi F., Falcinelli C., Forabosco A., Volpe A.: "Prenatal diagnosis of female pseudohermaphroditism associated with bilateral luteoma of pregnancy". *Hum. Reprod*., 2002, *17,* 821.

[9] Wang Y.C., Su H.Y., Liu J.Y, Chang F.W., Chen C.H.: "Maternal and female fetal virilization caused by pregnancy luteomas". *Fertil. Steril.,* 2005, *84*, 509. e15.

[10] Ugaki H., Enomoto T., Tokugawa Y., Kimura T.: "Luteoma-induced fetal virilization". *J. Obstet. Gynecol. Res.,* 2009, *35,* 991.

[11] Kao H.W., Wu C.J., Chung K.T., Wang S.R., Chen C.Y.: "MD1MR imaging of pregnancy luteoma: a case report and correlation with the clinical features". *Korean J. Radiol.*, 2005, *6*, 44.

[12] Chen C.H., Chen I.C., Wang Y..C, Liu J.Y., Wu G.J., Tzeng C.R.: "Boy born after gender preselection following successive gestational androgen excess of maternal luteoma and female disorders of sex development". *Fertil. Steril.,* 2009, *91*, 2732. e5.

[13] Manganiello P.D., Adams L.V., Harris D., Ornvold K.: "Virilisation during pregnancy with spontaneous resolution postpartum: a case report and review of English literature". *Obstet. Gynecol.,* 1995, *50,* 404.

[14] McClamrock H.D., Adashi E.Y.: "Gestational hyperandrogenism". *Fertil. Steril*., 1992, *57,* 257.

[15] Wang P.H., Chao H.T., Lee R.C., Lai C.R., Lee W.L., Kwok C.F.: "Steroid cell tumors of the ovary: clinical, ultrasonic, and MRI diagnosis- a case report". *Eur. J. Radiol.*, 1998, *26,* 269.

[16] Mok J.E., Shon W.S.: "Surgical management of steroid cell tumors of the ovary". *CME J Obstet Oncol.,* 2003, *8,* 173.

[17] Varras M., Vasilakaki T., Skafida E., Akrivis C.: "Clinical, ultrasonographic, computed tomography and histopathological manifestation of ovarian steroid cell tumor, not otherwise specified: our experience of a rare case with female virilisation and review of the literature". *Gynecol. Endocrinol.,* 2011, *27,* 412.

[18] Deavers M.T., Malpica A., Ordonez N.G. *et al.*: "Ovarian steroid cell tumors: an immunohistochemical study including a comparison of calretinin with inhibin". *Int. J. Gynecol. Pathol.,* 2003, *22,* 162.

[19] Taylor H.B., Norris H.J.: "Lipoid cell tumors of the ovary". *Cancer,* 1967, *20,* 1953.

Address reprint requests to:
K. HASEGAWA, M.D.
Department of Obstetrics and Gynecology
Fujita Health University School of Medicine
1-98 Dengakugakubo, Kutsukake-cho Toyoake,
Aichi, 470-1192 (Japan)
e-mail: gyne606@fujita-hu.ac.jp