Case 13
Dysgerminoma

Clinical History: The patient was 17 years old. A right adnexal mass was discovered and the patient was taken to surgery where a large right ovarian tumor was removed. There was no evidence of extraovarian tumor spread and the contralateral ovary was grossly normal.

Gross Pathology: The tumor replaced the ovary. It measured 17.0 cm in maximum diameter and weighed 1040 gm. The external surface was smooth and glistening. Cut sections revealed a solid tan tumor that was firm and rubbery.

Diagnosis: Dysgerminoma of the ovary.

Dysgerminoma

Case 13 provides an introduction to the topic of germ cell tumors of the ovary. These are uncommon tumors, but most pathologists are at least somewhat familiar with them because ovarian and testicular germ cell tumors are histologically similar, and surgical pathologists see the testicular tumors with some frequency.

Dysgerminoma is one of the two most common malignant germ cell tumors of the ovary. (1, 2) Still, it accounts for only 1-2% of all malignant ovarian tumors. Dysgerminoma occurs mainly in children and young women. (3-5) The average age is 22 years, and 90 percent of patients are less than 30 years of age. About 20 percent of malignant ovarian tumors detected during pregnancy are dysgerminomas. The usual presentation is with nonspecific findings such as abdominal distention, an abdominal mass, or abdominal pain. Some patients have menstrual abnormalities or gastrointestinal or urinary symptoms. Rare patients have hypercalcemia. (6) Serum lactic dehydrogenase (LDH) is frequently elevated but increased levels of serum alpha-fetoprotein or human chorionic gonadotropin are generally not detected. If increased, they suggest that other germ cell elements are present in the tumor. Of note, however, about 3% of patients with a pure dysgerminoma have increased amounts of beta-hCG in the blood, secreted by syncytiotrophoblastic cells within the tumor. (7) Dysgerminoma is the most common malignant gonadal tumor in patients with gonadal dysgenesis.
Dysgerminoma is confined to the ovaries (stage I) at diagnosis in 60–80 percent of patients. It is usually unilateral, which is characteristic of all malignant germ cell tumors. Dysgerminoma is unique among these tumors in that it is the only one with a significant incidence of bilaterality; both ovaries contain tumor (stage IB) in 5–15 percent of cases. The tumor in the contralateral ovary is grossly visible in half of the bilateral cases and it is a strictly microscopic finding in the other half. Some oncologists recommend biopsy of an apparently normal contralateral ovary if treatment is to be by unilateral salpingo-oophorectomy only. Dysgerminoma metastasizes via the lymphatics to the paraaortic lymph nodes, with subsequent spread to the mediastinal lymph nodes, and by transperitoneal spread to the pelvic and abdominal peritoneum. (8)

Unilateral encapsulated dysgerminoma (FIGO stage IA) can be treated by salpingo-oophorectomy with a 5-year survival rate of greater than 90 percent. (9) Postoperative therapy has been advocated for patients with localized disease, but there is an increasing trend to follow such patients closely and administer chemotherapy only to those who develop a recurrence. (10, 11) Recurrences can usually be successfully managed. When dysgerminoma develops in a dysgenetic gonad, the appropriate treatment is bilateral gonadectomy. The standard treatment for advanced disease (stage >IA) is total abdominal hysterectomy, bilateral salpingo-oophorectomy, limited debulking, and postoperative chemotherapy or radiotherapy. If they are not involved by tumor, the uterus and the contralateral ovary may be conserved in young patients when preservation of fertility is important. (12) Chemotherapy with platinum-based regimens is highly effective against dysgerminoma and is less likely than radiation to cause ovarian failure and infertility. Overall survival of optimally treated patients now exceeds 90 percent. (2, 9) Recurrences usually become evident within two years of primary treatment.

**Gross Pathology**

Dysgerminoma is a large solid tumor, usually more than 10 cm in diameter. It has a smooth outer surface and the cut surface tends to be fleshy, homogeneous or nodular, and gray, tan, or white. Hemorrhage and necrosis are often present, especially in large tumors.

**Microscopic Pathology**

Dysgerminoma is composed of polygonal cells with abundant granular eosinophilic or clear cytoplasm and distinct cell membranes. As shown in Fig. 13-1, the nuclei are round, medium sized and relatively uniform with vesicular chromatin and prominent nucleoli. Mitotic figures are usually numerous. The cells grow in sheets, nests or trabeculae that are separated by fibrous septa, as shown in Fig. 13-2. The septa vary from thin wisps of connective tissue to thick fibrous bands. Lymphocytes are usually present in the stroma and in lesser numbers among the tumor cells. Most of the lymphocytes are T-cells, particularly those among the tumor cells, with B-cells mainly confined to the septa. (13, 14) When numerous lymphoid cells are present in the stroma germinal centers may be present. Epithelioid cells and
multinucleated Langhans type giant cells are present in many dysgerminomas in loose aggregates or sarcoid-like granulomas.

The diagnosis of dysgerminoma is straightforward in typical cases, but there are a number of variations in appearance that can cause diagnostic problems, particularly in small biopsies or frozen sections. Necrosis is occasionally so extensive that it obliterates so much of the underlying tumor that it is difficult to recognize. Focal or extensive fibrosis can be present in dysgerminoma, and can overshadow the tumor cells. I have never seen a case in which the tumor is completely replaced by fibrous tissue, as sometimes occurs in testicular seminomas. When granulomatous inflammation is very extensive it may be difficult to see the tumor cells among the epithelioid cells. Edema and loss of intercellular cohesion sometimes result in formation of gland-like spaces or microcysts, a pattern that has been called “tubular” dysgerminoma. Dysgerminomas with marked nuclear atypia and increased mitotic figures (more than 30 mitotic figures per 10 high power fields) dysgerminoma can mimic embryonal carcinoma. Such tumors were called “anaplastic” dysgerminomas in the past, but since the higher grade histology is not correlated with more aggressive clinical behavior they are now simply viewed as a variant histologic pattern of dysgerminoma. Poor fixation can result in clumping of the nuclear chromatin and dense basophilic cytoplasm, raising the question of embryonal carcinoma. About 3% of dysgerminomas contain syncytiotrophoblastic giant cells. (7) When STGC are present, the patient can have a positive pregnancy tests and/or elevated serum levels of beta-human chorionic gonadotropin. Dysgerminomas with STGC lack cytotrophoblastic cells and no other germ cell elements are present so these tumors are classified as variants of dysgerminoma, not as choriocarcinoma or as a mixed germ cell tumor. Although dysgerminoma variants can be difficult to diagnose, there is no evidence that their clinical behavior is any different than that of typical dysgerminomas. The correct diagnosis can usually be established by study of additional slides that reveal more typical histologic features or by performing immunohistochemical stains.

Fig. 13-2. Trabecula with lymphocytes

<table>
<thead>
<tr>
<th>Extensive necrosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extensive fibrosis</td>
</tr>
<tr>
<td>Extensive granulomatous reaction</td>
</tr>
<tr>
<td>Alveolar, tubular or microcystic pattern</td>
</tr>
<tr>
<td>Anaplastic tumor cells and/or high mitotic rate</td>
</tr>
<tr>
<td>Poor fixation artifacts</td>
</tr>
<tr>
<td>Syncytiotrophoblastic giant cells</td>
</tr>
</tbody>
</table>
Molecular Pathology and Immunohistochemistry

Chromosome 12p abnormalities are present in many malignant germ cell tumors. The most common abnormality is an isochromosome, i12p, but over-representation of chromosome 12p material is sometimes found present in addition to or instead of an i12p. In one study, an i12p was identified in 16 of 21 dysgerminomas and over-representation of chromosome 12 material was detected in 5 dysgerminomas. (15) c-KIT mutations have been identified detected in about 25% of dysgerminomas, but they are located in exon 17, not the exon 11 location that confers sensitivity to imatinib. (16, 17) There is no correlation between the presence or absence of a c-KIT mutation and immunohistochemical staining for CD117.

Numerous immunohistochemical stains are available to confirm a diagnosis of dysgerminoma. These fall into two main groups: antibodies against cytoplasmic and membranous antigens and antibodies against nuclear antigens. The first group includes placental alkaline phosphatase (PLAP), CD117 (c-kit) and D2-40. (18, 19) All are excellent markers for dysgerminoma. PLAP was the first of these markers to be introduced and is the most widely available, but I prefer the newer markers CD117 and D2-40. Staining for PLAP and limited staining for D2-40 can be seen in other types of germ cell tumors. Cytoplasmic staining is often present but only membrane staining is considered to be indicative of dysgerminoma. Remember that cytoplasmic staining for CD117 is seen in some tumors that can mimic dysgerminoma, such as melanoma, but membrane staining is characteristic of dysgerminoma. In general, staining is strongest for D2-40, while CD117 is least likely to stain necrotic background debris. The second group of immunostains, those for nuclear antigens, includes OCT-4, NANOG, and SALL4 (Figs. 4c and 4d). (16, 18, 20, 21) These are nuclear transcription factors that are present in primitive germ cell tumors. Positive staining is not limited to dysgerminoma; all three are positive in embryonal carcinoma and SALL4 also stains yolk sac tumor. Diffuse strong nuclear staining is characteristic of dysgerminoma but a definitive diagnosis requires a panel of stains that enable the pathologist to identify the specific types of germ cell neoplasia that are present. Immunostains for cytokeratin typically show very focal cytoplasmic dot or rim-like staining in dysgerminoma; the type of diffuse strong cytoplasmic or membranous staining that is seen in embryonal carcinoma and yolk sac tumor is not present. (22) Dysgerminoma is generally negative for epithelial membrane antigen (EMA). (22) Since many of the positive markers for dysgerminoma lack specificity a panel of stains, usually including CD117 and/or D2-40, OCT4 and/or SALL4 and cytokeratin must be used to confirm the diagnosis. Immunostains for hCG are generally negative, but the syncytiotrophoblastic giant cells that are present in dysgerminomas from patients with elevated serum beta-hCG show positive cytoplasmic staining. Immunostains for α-fetoprotein (AFP) are negative.
Differential Diagnosis

Many clear cell tumors occur in or spread to the ovaries, as detailed in the discussion of Case 3. A number of them enter the differential diagnosis of dysgerminoma, including other types of malignant germ cell tumors and various types of non-germ cell tumors can also be confused with dysgerminoma. Dysgerminoma is the most common component of mixed germ cell tumors of the ovary, so it is important to search carefully to exclude other germ cell elements before making a diagnosis of a pure dysgerminoma.

Malignant mixed germ cell tumor
   Embryonal carcinoma
   Yolk sac tumor
   Clear cell carcinoma
   Lymphoma
   Melanoma

Embryonal carcinoma is mainly seen as a minor component in mixed germ cell tumors and only infrequently occurs in pure form in the ovary. If it is extensive and the cells grow in solid sheets embryonal carcinoma can resemble dysgerminoma, particularly if the cytoplasm is pale or clear. Also, as previously mentioned, anaplastic variants of dysgerminoma can be confused with embryonal carcinoma. Embryonal carcinoma cells have larger more pleomorphic nuclei and the chromatin is coarser. The cytoplasm is most often amphophilic or basophilic rather than clear or eosinophilic. Neither granulomas nor a lymphoid stroma are present in embryonal carcinoma. Careful evaluation often reveals poorly formed glands. There is positive staining for the nuclear transcription factors OCT4, NANOG, and SALL4 in both embryonal carcinoma and dysgerminoma so these stains do not differentiate the two. Dysgerminoma is usually strongly positive for CD117 and D2-40, while embryonal carcinoma is generally strongly positive for CD30 and cytokeratin. The anti-cytokeratin antibody AE1/AE3 shows a membranous staining pattern in embryonal carcinoma. Strong keratin staining excludes dysgerminoma.

Yolk sac tumor grows in a confusing variety of patterns, most of which are unlikely to raise the question of dysgerminoma. A solid pattern of growth of cells with clear cytoplasm can, however, resemble dysgerminoma. The tumor cell nuclei tend to be smaller and darker in yolk sac tumor, and the overall cell size is also smaller. Thorough study generally reveals additional more characteristic patterns of yolk sac tumor. Immunostains are helpful in this differential diagnosis. Both dysgerminoma and yolk sac tumor are positive for SALL4, but yolk sac tumor does not stain for OCT4 or NANOG, nor have we observed membranous staining for CD117 or D2-40. Staining for PLAP is less extensive in yolk sac tumor than in dysgerminoma. Yolk sac tumor shows diffuse strong cytoplasmic staining for cytokeratin, and most examples are positive for alpha-fetoprotein and glypican-3, which are negative in dysgerminoma. Knowledge of the clinical presentation is helpful, as patients with yolk sac tumor generally have an elevated serum alpha-fetoprotein.
Clear cell carcinoma patients are usually older than those with dysgerminoma, and endometriosis is often found in patients with clear cell carcinoma. The solid pattern of clear cell carcinoma can mimic dysgerminoma, but the tumor cell nuclei are larger. Most clear cell carcinomas also exhibit areas of tubulocystic and papillary growth. Immunostains for cytokeratin and EMA are strongly positive in clear cell carcinoma, but are negative or only focally and weakly positive in dysgerminoma. Clear cell carcinoma is negative for most dysgerminoma stains discussed above, PLAP being the only one that might show staining in a carcinoma.

Lymphoma can resemble dysgerminoma because it is a monotonous proliferation of cells with medium sized nuclei and the cell cytoplasm can be clear or pale. Lymphoma often surrounds residual ovarian structures, rather than displacing them, as occurs in dysgerminoma. Dysgerminoma cell nuclei tend to be vesicular, while the nuclear chromatin is often coarsely granular in lymphoma and lymphoma cells usually have less cytoplasm. Most lymphomas of the ovary are B-cell lymphomas, and accordingly stain for leukocyte common antigen and various markers of B-cell differentiation, all of which are negative in dysgerminoma. Lymphomas do not stain for dysgerminoma markers.

Melanoma of the ovary is most often metastatic, but it can be primary, arising in a teratoma. The histologic appearance is variable, and melanoma can mimic a variety of neoplasms, including dysgerminoma. Melanoma often exhibits a variety of growth patterns and the tumor cell nuclei tend to be pleomorphic while dysgerminoma is monotonous, so examination of an adequate number of sections generally differentiates melanoma from dysgerminoma. Melanoma cells sometimes contain visible brown melanin pigment. Immunostains for melanoma markers such as S100, SOX10, HMB45 and Melan A are almost always positive in melanoma, but negative in dysgerminoma. Dysgerminoma markers are negative in melanoma, although cytoplasmic, but not membranous, staining for CD117 occurs in melanoma.

References

Case 14
Immature Teratoma

Clinical History:

TR64-13818: The patient was a 16 year old girl. Four weeks prior to admission she developed abdominal pain and rebound tenderness, suggestive of peritonitis. She had a mild fever. Antibiotics were administered and the discomfort slowly diminished and the temperature returned to normal. There was a suggestion of enlargement of the right ovary. She was seen 3 weeks later at which time a physician palpated a large mass extending almost to the umbilicus. The patient was taken to surgery and a large mass of the left ovary was found and removed.

TR90-26689: The patient was a 30 year old woman who presented with a 1-week history of pelvic pain. At surgery, a large right ovarian tumor was found and removed.

Gross Pathology:

TR64-13818: A large ovarian tumor measuring 12.0 cm in diameter was removed. It had a smooth glistening external surface. The cross sections showed a large cyst and several smaller cysts with solid masses measuring up to 7.0 cm in diameter projecting into the lumens. A few hairs grew from one of the smaller masses into one of the cysts. The cut surfaces had areas of hemorrhage and there were small foci of calcification.

TR90-26689: The right ovary measured 15 cm in maximum dimension. It was predominantly solid with rubbery white tan cut surfaces. There were multiple cysts ranging from 0.2 to 10 cm in maximum dimension. Brown hair was noted and there were focal gritty areas of calcification. There were occasional foci of hemorrhage and necrosis. A biopsy of a bowel adhesion showed fibrosis, but no tumor.

Diagnosis: Immature Teratoma, with microscopic foci of yolk sac tumor in TR64-13818

Teratoma

A type of teratoma, the benign cystic teratoma (dermoid cyst), is the most common ovarian neoplasm, accounting for more than a quarter of all ovarian tumors. (1, 2) Most teratomas have a 46XX karyotype and appear to be derived from postmeiotic germ cells. There are numerous benign and malignant variants of teratoma of the ovary. Those in which one element greatly predominates are termed “monodermal” teratomas.

Immature Teratoma

Immature teratoma is the most common malignant germ cell tumor of the ovary, representing 20-35% of such tumors at major cancer centers (3, 4) and in the U.S. population. (5) Immaturity in teratomas has been linked to stages of fetal development. An immature teratoma contains at least some tissue of a type seen prior to a fertilization age of 8 weeks while a mature teratoma consists exclusively of tissues similar to those seen at a fertilization age of 8 weeks or more. (6) High-grade immature teratoma is characterized by the presence of immature neuroepithelial structures, and low-grade immature teratoma by the presence of somite organogenesis.
Clinical Features

Like other malignant germ cell tumors, immature teratoma occurs predominantly in children and young women. Patients are rarely younger than 7 or older than 40. The average patient age is about 20 years. While it is uncommon, immature teratoma occasionally occurs in an older or postmenopausal woman. (7)

The clinical presentation is generally nonspecific with pelvic or abdominal pain, abdominal distention, or a palpable abdominal mass. Occasional patients have acute abdominal symptoms caused by infarction or rupture of the tumor. A rare but dramatic presentation is with the sudden onset of severe psychiatric or neurologic symptoms, including coma (“teratoma coma”) in some cases. The symptoms are the result of a paraneoplastic encephalitis that is a form of autoimmune disease caused by antibodies to anti-N-methyl-D-aspartate receptors (anti-NMDAR). Neural tissues in the teratoma are thought to initiate development of the anti-NMDAR antibodies. Treatment includes removal of the teratoma followed by immunotherapy. (8, 9) Alpha-fetoprotein is elevated in the serum in many patients with pure immature teratoma. (10, 11) CA 125 is also frequently elevated, albeit not to the levels seen in patients with serous tumors of the ovary. (10)

Most (50-80%) patients have localized tumors (stage I) at diagnosis. Immature teratoma is almost always unilateral, although spread to the contralateral ovary can occur in patients with advanced disease. A benign cystic teratoma is present in the contralateral ovary in 10-15% of cases. Immature teratoma spreads mainly within the abdomen to the peritoneum and the omentum.

Since immature teratoma occurs mainly in young patients, treatment is generally as conservative possible, with the goal of preserving fertility. Patients whose tumors are confined to the ovary (stage IA) are generally treated by unilateral salpingo-oophorectomy. A few have even been successfully treated by cystectomy, sometimes followed by adjuvant chemotherapy. (12) Advanced tumors in young patients are treated by unilateral salpingo-oophorectomy and excision of extra-ovarian tumor. (4, 13) Hysterectomy and bilateral salpingo-oophorectomy is the usual treatment for older patients and those with extraovarian tumor spread involving the contralateral ovary or the uterus.

Immature teratoma is unique among the malignant germ cell tumors in that treatment is based not only on the tumor stage, but also on the tumor grade. Patients with stage IA grade 1 tumors have an excellent prognosis after surgery and usually do not receive chemotherapy. (4, 14) Adult patients with localized grade 3 immature teratomas and those who have advanced disease require postoperative chemotherapy. Whether or not adult patients with stage IA grade 2 tumors should have chemotherapy is controversial, but the current NCCN guidelines suggest that these patients be treated. In children, the prognosis appears to be more favorable and independent of tumor grade. (15) Children accordingly generally do not have chemotherapy unless they have metastases containing other malignant germ cell elements such as yolk sac tumor. (11, 16) Cisplatin-containing regimens such as BEP (cisplatin, etoposide, and bleomycin) are highly effective as adjuvant chemotherapy for patients with no residual tumor after surgery, with survival rates of 90-100%. (5, 17) The prognosis is less favorable when there is residual gross tumor after primary surgery and in cases of recurrent immature teratoma. (18)

The microscopic appearance of extraovarian tumor deposits is important in determining whether additional therapy is necessary. Sometimes the extraovarian tumor deposits, which are usually in the omentum, on the peritoneum, or in lymph nodes, consist entirely of mature tissues, most often glial predominant neural tissue. Such completely mature deposits are graded as grade
0. Grade 0 tumor deposits do not adversely affect the prognosis, and do not require chemotherapy. A stage > I is assigned only if the extraovarian tumor is immature. Masses composed of mature teratoma are sometimes detected after chemotherapy in patients with incompletely resected immature teratoma. They are generally resected to prevent local complications and to avoid development of a “growing teratoma syndrome.” (19, 20) We have seen small grade 0 tumor deposits that have persisted for more than 40 years after resection of an ovarian teratoma and caused no signs or symptoms. Rare examples of a malignant tumor arising in long-standing incompletely resected low-grade teratoma implants have been reported. (21-23)

**Gross Pathology**

Immature teratoma generally has an obviously different appearance than the usual benign teratoma, as it is mostly solid. It is a unilateral tumor that varies in size; the average diameter is 18 cm. Solid areas within the tumor are white, tan, gray or brown and can be soft or firm. If cartilage or bone is present the tumor is hard and gritty. Scattered small cysts are typically present and about 25% of immature teratomas have large cysts that contain keratinous debris or hair, as seen in a benign cystic teratoma. (24)

**Microscopic Pathology**

Tissues derived from any of the three germ cell layers can be present. Immature tissues are most often of ectodermal and mesodermal origin. A mixture of mature and immature tissues is present in most tumors, with mature elements usually greatly predominating. Immature neuroectodermal elements are the dominant immature element, and they are also the easiest immature tissue to recognize and quantitate for purposes of grading (Fig. 14-1). (25, 26) Immature neuroectodermal tissues include neuroepithelial tubules or trabeculae lined by mitotically active columnar cells with stratified hyperchromatic nuclei (Fig. 14-2), sheets and nests of neuroblasts, sometimes containing neuropil and Homer Wright rosettes, hypercellular mitotically active immature glia, and primitive retinal tissue with melanin pigmentation. (27, 28) Prominent but benign vascular proliferations are occasionally associated with the neural elements in an immature teratoma. (29) Rosettes lined by one or two layers of ependymal cells are present in some benign teratomas; they should not be mistaken for the primitive rosettes seen in an immature teratoma. The cells lining ependymal rosettes have ample cytoplasm, the nuclei form only one or two layers, and mitotic figures are not present.

Immature mesodermal tissue is hypercellular and consists of small spindle cells with hyperchromatic nuclei. Mitotic figures are usually present. Immature cartilage is often present in
immature teratomas. Two main features distinguish it from the more common “fetal” or “mature” cartilage that is often present. First, chondroid cells within the lacunae have medium sized round nuclei with open chromatin, as opposed to the small nuclei with dark chromatin seen in fetal cartilage. Second, foci of immature cartilage are surrounded by primitive small round mesenchymal cells rather than by the fibroblasts that typically surround mature cartilage.

Endodermal tissues are usually less extensive than ectodermal or mesodermal tissues in an immature teratoma, and are only infrequently the predominant type of immature tissue present. Immature endodermal tissues that can be seen include primitive glands lined by columnar cells with subnuclear and supranuclear vacuoles resulting in an “enteroblastic” appearance, glands lined by partially differentiated stratified columnar intestinal epithelium with goblet cells, and islands of fetal liver tissue. (30, 31) Pediatric pathologists have proposed that foci of immature liver and glands with subnuclear vacuoles similar to immature endoderm or fetal lung are well differentiated forms of yolk sac tumor, (15) although gynecologic pathologists tend to view limited amounts of these elements as components of the teratoma. (30) Immature renal (metanephrogenic) tissue is another rare type of immature tissue that can be seen in teratomas. (32)

Immature teratoma is graded from grade 1 for a neoplasm composed almost entirely of mature tissues to grade 3 for a neoplasm containing easily detected immature tissue. The current grading system is based on the amount of immature neuroepithelium present. (26) A two-grade system in which grade 1 tumors are designated as low grade and grades 2 and 3 as high grade was proposed but it is not widely used. (33)

<table>
<thead>
<tr>
<th>Grade</th>
<th>Amount of Immature Tissue</th>
<th>Amount of Immature Neuroepithelium</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>Rare, not &gt; 1 LPF/slide</td>
</tr>
<tr>
<td>2</td>
<td>++</td>
<td>Common, but not &gt; 3 LPF/slide</td>
</tr>
<tr>
<td>3</td>
<td>+++</td>
<td>Prominent, ≥ 4 LPF/slide</td>
</tr>
</tbody>
</table>

LPF = Low power field (40x, 4x objective, 10x eyepiece)

Occasionally, a mature teratoma contains only one or a few microscopic foci of immature tissue. There is very limited information about the outcome for patients with such tumors, but a in a study of 10 mature cystic teratomas with microscopic foci of immature tissue no patient had a recurrence. (24) Follow-up was available in 9 of the 10 cases ranging from 11 months to 7 years. When only a miniscule amount of immature tissue is present in an otherwise mature ovarian teratoma, I make a diagnosis of a benign teratoma with a microscopic focus of immature tissue and suggest that it may be most appropriate for the patient to have clinical follow-up rather than additional therapy.

Microscopic foci of yolk sac tumor are occasionally detected in an immature teratoma. These foci tend to be subtle and often go unrecognized. Several are present in one of the cases used in this seminar. Their presence suggests that the tumor developed via a primitive germ cell pathway, by maturation from embryonal carcinoma, as discussed above. As long as there are only a few microfoci of yolk sac tumor (≤ 3) and they are less than 3 mm in diameter they do not appear to adversely impact the prognosis. (33) If they are larger or numerous, a diagnosis of mixed germ cell tumor would be appropriate.
Deposits of teratoma are not uncommonly detected in the omentum, on the peritoneum, and in lymph nodes in patients with immature teratoma of the ovary. Immature tissue is occasionally present in such implants, but they most often consist predominantly of mature neural tissue and the deposits are accordingly often designated as “gliomatosis.” (34) Similar tumor deposits also occasionally occur in patients with a mature teratoma. The deposits are typically not composed only of glial cells; neurons and neurofilaments are also frequently present and other benign mesenchymal and epithelial elements are sometimes seen as well. Florid vascular proliferations like those that occasionally occur in immature teratoma in the can also be seen in the implants. (35) When only mature tissues are present in an implant it is designated as grade 0. Occasional implant consist of an admixture of mature teratomatous elements and endometriosis. (36, 37) The implants were long thought to represent a form of extraovarian spread from the teratoma but recent molecular pathology studies have shown genetic differences between grade 0 tumor deposits and the primary ovarian tumors. Instead, the deposits have a genetic pattern similar to normal tissue from the patient. (38, 39) In another study, multiple omental and peritoneal implants were analyzed and found to have mutually exclusive genetic differences. (40) These findings suggest to some that the extraovarian tumor deposits must represent a form of metaplasia rather than metastases from the ovarian tumor, a conclusion that is not universally accepted. (41)

**Immunohistochemistry and Molecular Pathology**

Genetic studies of a limited number of cases have revealed that an isochromosome 12p, which is characteristic of primitive germ cell tumors, is often present in the immature teratoma component of a mixed germ cell tumor, (42) compatible with its derivation from a less differentiated component of the tumor. On the other hand, an i12p is typically not present in pure immature teratomas, suggesting a different pathogenesis for such tumors. (42, 43) The histogenesis of immature teratomas is still unsettled however, since immature teratoma is typically diploid, whether it is found as a pure tumor or as a component of a mixed germ cell tumor, while other germ cell elements such as yolk sac tumor are aneuploid, (44, 45) and an i12p has been detected in a pure immature teratoma. [Rogriguez, 1995]

Immunohistochemistry is not as widely used in the diagnosis of immature teratoma as it is in the diagnosis of other types of malignant germ cell tumors. In most cases, the diagnosis and grade are based on the appearance of the tumor on hematoxylin and eosin stained slides. It has been proposed that expression of glial cell line-derived neurotropic factor receptor alpha-1 (GFRalpha-1) might be useful for the identification of immature neuroepithelium, (46) but this antibody is not widely available, and is usually not needed. Primitive neuroepithelium may stain for CD99 and bcl-2, and immature cartilage for CD34 and bcl-2. (6) Staining for glial fibrillary acidic protein (GFAP) can help identify glial differentiation. Primitive neuroepithelial cells do not stain with GFAP, but they sometimes stain for neurofilaments or neuron specific enolase (NSE). (28, 47) Intestinal and respiratory epithelium contains argyrophilic cells that stain for synaptophysin and chromogranin and with antibodies to a variety of neurohormonal peptides.

Some of the tissues in immature teratomas can show staining with antibodies generally thought of as markers of more primitive germ cell tumors; this can complicate identification of primitive elements in a mixed germ cell tumor. For example, alpha-fetoprotein, generally used as a marker for yolk sac tumor, shows positive staining in immature liver and immature endodermal glands. (6, 30, 48) Foci of immature liver can also be confirmed with immunostains for anti-hepatocyte antibody (Hepar) and arginase. SALL4, a marker for embryonal carcinoma,
dysgerminoma, and yolk sac tumor, SOX2, a marker for embryonal carcinoma, and glypican-3, a marker for yolk sac tumor, all show occasional staining in immature teratomas. Staining can be seen in immature neuroepithelium, blastomatous stroma, liver and immature glands. (49-51) Elevated levels of serum alpha-protein are sometimes detected in patients with immature teratomas. This does not necessarily indicate the presence of yolk sac tumor, as immature liver and immature endodermal glands can be AFP positive and thus may be the source of the AFP in the serum.

It can be tricky to identify microfoci of yolk sac tumor in an immature teratoma. Such foci can be detected on H&E stained slides, but they can be difficult to confirm with immunohistochemistry because they do not always stain for AFP. Staining for AFP can be weak and focal, so when very limited amounts of yolk sac tumor are present they may be AFP negative. Newer stains, such as SALL4 and glypican-3 are more sensitive than AFP and they are generally positive in microfoci of yolk sac tumor, as in this case. Other stains that can be helpful include HNF-1, which is positive in yolk sac tumor cell nuclei, and keratin, which typically shows cytoplasmic staining in yolk sac tumor.

**Differential Diagnosis**

It is sometimes difficult to decide whether a teratoma is immature or mature. The question of whether a teratoma that has only a miniscule amount of immature tissue should be classified as an immature teratoma is discussed above; our approach is to classify such tumors as mature teratomas with microscopic foci of immature tissue. (24) Sometimes it is not clear whether a component of a teratoma should be considered to be immature or mature. One approach is to view any tissue type that could be seen in fetal development after a fertilization age of 8 weeks as “fetal” type tissue indicative of a mature teratoma. (6) A few specific findings seem especially likely to cause diagnostic problems. Ependymal tubules are occasionally seen in mature teratomas and can be mistaken for immature neuroepithelial tubules. However, they are lined by a single layer of cells, show no mitotic activity, and show minimal staining for proliferation markers such as MIB-1. Cerebellar type tissue is sometimes detected in mature teratomas. A fetal external granular layer can be present in this tissue and should not be mistaken for the primitive neuroectodermal cells of an immature teratoma. Glial tissue in mature teratomas can be surprisingly cellular but unless it is hypercellular and mitotically active it should be viewed as compatible with a mature teratoma. (27) The fetal type cartilage seen in mature teratomas consists of hyaline matrix with chondrocytes in lacunae. The chondroid foci are surrounded by fibrous stroma. Embryonal cartilage, which is indicative of an immature teratoma, consists of chondroid matrix containing plump chondroblasts with pale round nuclei. This type of cartilage is surrounded by cellular immature stroma.

*Malignant mixed germ cell tumor* is a neoplasm that contains two or more malignant germ cell elements. Immature teratoma is a frequent constituent of such tumors so whenever it is identified a careful search for other malignant germ cell elements is mandatory. Generally, this requires evaluation of one block per cm of tumor diameter. Microfoci of yolk sac tumor are present in 5 to 10% of immature teratomas. (33) As long as they are small (< 3mm) and there are only a few of them, they do not warrant designating the tumor as a mixed germ cell tumor, although their presence should certainly be noted in the pathology report. Larger foci of yolk sac tumor are indicative of a mixed germ cell tumor.
**Mixed mesodermal tumor** or **carcinosarcoma** is a high grade neoplasm that could potentially be mistaken for an immature teratoma. This is because carcinosarcoma, like teratoma, contains a mixture of epithelial and mesenchymal tissues. (47) However, carcinosarcoma is a tumor of older women, outside of the age range of immature teratoma. It is entirely of mesodermal derivation and is composed of a mixture of carcinoma and sarcoma, not the immature elements seen in an immature teratoma. Neither ectodermal or endodermal elements are present. The epithelial component can be any type of ovarian carcinoma but endometrioid, serous, squamous cell, and undifferentiated carcinoma patterns are most common. Likewise, the mesenchymal component can be any type of homologous or heterologous sarcoma with a nonspecific fibrous sarcoma and rhabdomyosarcoma being the most common types of homologous and heterologous sarcomatous elements.

**Primitive neuroectodermal tumor (PNET)** is a rare variant of immature teratoma that contains neuroectodermal cells growing in patterns reminiscent of various tumors of the central nervous system. Tumors of this type also occur in the testis where they are often associated with teratomas. The diagnosis of neuroectodermal tumor of the testis is made when the neuroectodermal component measures at least 1 cm in diameter.(52) It seems reasonable to apply this size standard to ovarian tumors as well, although, in ovarian tumors, the neuroectodermal component generally comprises most or all of a large neoplasm, overgrowing the underlying teratoma. Primitive neuroectodermal tumors consist of nests and sheets of small cells with hyperchromatic mitotically active nuclei.(53-55) Some cells have fibrillary cytoplasm and some tumors contain rosettes with central lumina, neuropil, neuroblastic rosettes, or foci of glial differentiation. PNETs can resemble medulloepithelioma, ependymoblastoma, medulloblastoma or neuroblastoma.

These tumors are generally classified separately, although they might also be viewed as high grade variants of immature teratoma. Rare examples of the peripheral type of PNET, a tumor in the Ewing sarcoma/PNET family have also been reported in the ovary. These can bear a resemblance to the more common central type of PNET, but they are not associated with other teratomatous elements, and they have the chromosomal translocation that characterizes tumors in the Ewing sarcoma/pPNET family. (56)

**References**

Case 15
Granulosa Cell Tumor

Clinical History: A 47 year old woman was operated on for “fibroids.” At surgery, the uterus contained leiomyomas, but there was also a 14.9 cm diameter left ovarian tumor. She had multiple peritoneal and lymph node biopsies and no tumor was identified outside the ovary.

Gross Pathology: The ovary contained a pink tan mass measuring 14.9 cm in maximum dimension. The exterior of the ovary was smooth and glistening, with no evidence of external tumor growth. The cut surfaces were fleshy and ranged from light tan to pink to tan-yellow. There were fibrous septa between lobules of tumor.

Diagnosis: Granulosa Cell Tumor

Follow-up: The patient was alive and well in November, 2011 (10 years after treatment), when she was last seen.

Granulosa Cell Tumor

1–2 percent of all ovarian tumors are granulosa cell tumors. (1) Granulosa cell tumor is the most common malignant sex cord-stromal tumor. There are two types of granulosa cell tumor: an adult type that occurs mainly in peri and postmenopausal women and a juvenile type that occurs mainly in children.

Adult Granulosa Cell Tumor

Adult granulosa cell tumors are most often detected in postmenopausal women, but they occur over a wide age range, from teenagers to the elderly. The average patient age is 45–55 years. Granulosa cell tumors characteristically secrete estrogens, which stimulate the endometrium to proliferate. The usual presenting symptom is postmenopausal bleeding in older women and menorrhagia, metrorrhagia, or amenorrhea in younger women. In the past, the endometrium was reported to be hyperplastic in 30–40% of patients and 5–10% had endometrial adenocarcinoma. (2-5) Based on my experience this is no longer the case. Proliferative types of endometrium are common, but hyperplasia is rarely seen these days, and I have never seen a case of endometrial carcinoma that I could attribute to a granulosa cell tumor. Most likely, earlier diagnosis these days prevents the development of more extreme types of endometrial pathology. Still, if the treatment is not going to include hysterectomy, the clinician should perform an endometrial biopsy to ascertain the status of the endometrium. Rare adult-type granulosa cell tumors, most occurring in young women 15–35 years of age, secrete androgens and cause the patient to become virilized. (6-11) Typical symptoms of virilization are hirsutism, enlargement of the clitoris, deepening of the voice and amenorrhea. About 25% of patients with granulosa cell tumors have presenting symptoms that are not hormone related. These include abdominal distention, pain, or a palpable mass. Rupture of the tumor or torsion with infarction and intratumoral hemorrhage can cause acute abdominal symptoms. Most women with a granulosa cell tumor have a palpable unilateral adnexal mass; bilateral tumors are uncommon.

The tumor is limited to the ovary (FIGO stage I) at diagnosis in 80–90% of cases. Most patients are older and the standard treatment is total abdominal hysterectomy and bilateral salpingo-oophorectomy. Unilateral salpingo-oophorectomy is suitable treatment for stage IA
tumors in young women who wish to conserve their fertility. All granulosa cell tumors have malignant potential, although most do not recur or metastasize. The recurrence rate is 10–15% for stage IA tumors and 20–30% overall. (4, 12-17) Extraovarian spread is to the peritoneum and omentum and occasionally to the liver or lungs. (18, 19) Lymph node metastases are uncommon so routine dissection of the pelvic and abdominal lymph nodes is unnecessary. (20, 21) When intra-abdominal spread is present at diagnosis (stage III) or the tumor recurs a majority of patients die of tumor. Granulosa cell tumors are indolent and metastases, if they develop, are often detected more than 5 years after initial treatment. There are many reports of disease-free intervals of more than 20 years. (22) Chemotherapy for advanced or recurrent granulosa cell tumor is less than optimal. Some patients respond to combinations of drugs that includes cisplatin, (23-26) but responses are seldom durable. One group reported that women who completed 6 full cycles of BEP chemotherapy were less likely to have additional recurrences. (27) Anti-angiogenesis therapy with bevacizumab has resulted in partial or complete remission in some patients. (28) The value of radiotherapy is unclear, but there is some evidence that in selected cases adjuvant radiotherapy can result in a longer disease free survival. (29)

Several potential tumor markers have been identified in the sera of women with granulosa cell tumors, including estradiol, müllerian inhibiting substance, follicle regulatory protein, and inhibin. Inhibin has emerged as the most widely used tumor marker, as serum inhibin levels are elevated in nearly all patients with primary or recurrent granulosa cell tumor. (30, 31) Inhibin is not specific for granulosa cell tumor, as elevated serum concentrations can be observed in women with other types of ovarian tumor, but once the diagnosis has been established it can be used for monitoring treatment and detecting recurrence.

**Gross Pathology**

Granulosa cell tumors vary in size from small incidentally discovered tumors only a few millimeters in diameter to large neoplasms more than 30 cm in diameter. The average size is about 10 cm. Some are totally solid, but most are partly cystic. The solid portions are pink, tan, brown, or light yellow and vary from soft to firm in consistency. Rare granulosa cell tumors are entirely cystic with a wall only a few millimeters thick. These seem to be more likely than other granulosa cell tumors to be androgenic. (7, 15)

**Microscopic Pathology**

The tumor cells resemble normal granulosa cells. They are small and round, cuboidal, or spindle-shaped with pale cytoplasm and ill-defined cell borders. The nuclei are round or oval with fine chromatin and a single small nucleolus (Fig. 15-1). Longitudinal folds or grooves are often present in the nuclei and are a characteristic feature of adult granulosa cell tumor. Numerous mitotic figures, nuclear pleomorphism and atypia are unusual findings, but may be present. Some tumors contain cells with bizarre nuclei, but this finding does not appear to impact the prognosis. (32-34) A rare finding, present in about 1% of cases, is extensive (> 50% of cells) luteinization of the tumor cells. Luteinized granulosa cells have abundant eosinophilic cytoplasm, well-defined cell borders, and central nuclei, and resemble the luteinized granulosa cells of the corpus luteum. A nodular pattern, as seen at low magnification, and myxoid background stroma are characteristic. Luteinized granulosa cell tumors occur in pregnancy, in patients with androgenic tumors, and as idiopathic findings. (6, 35, 36)
Various histologic patterns have been described in granulosa cell tumors. These are typically mixed and do not have and prognostic significance, but they help identify a tumor as a granulosa cell tumor. The microfollicular pattern is the most characteristic one and consists of nests and sheets of granulosa cells in which there are small spaces containing eosinophilic secretions and cellular debris (Fig. 15-2). The spaces resemble the Call–Exner bodies of developing follicles. In the macrofollicular pattern there are large rounded or irregularly shaped follicles lined by stratified granulosa cells. The macrofollicular pattern is seen most often in juvenile granulosa cell tumors. The tumor cells grow in anastomosing bands, ribbons, and cords in the trabecular pattern; as closed or even open tubules in the tubular pattern; in irregular undulating ribbons in the gyriiform or watered-silk pattern; and in nests and islands in the insular pattern. There are large irregular sheets of tumor cells with no organized substructure in the solid or diffuse pattern. Small or large cysts lined by one or more layers of granulosa cells are often present; they frequently contain blood and hemosiderin-laden macrophages are often present in the cysts and the cyst wall. Rare tumors grow as large unilocular cysts lined by stratified granulosa cells, among which microfollicles or trabecular growth are present. Occasionally, there are areas in cystic granulosa cell tumors where the tumor cells line blunt or branching papillae that project into cystic spaces; this finding is more common in juvenile granulosa cell tumors than in adult type tumors. (37)

Granulosa cell tumors have a variable amount of fibrous or thecomatous stroma. Tumors with abundant fibrothecomatous stroma were formerly designated as granulosa-theca cell tumors. Currently, any tumor in which granulosa cells comprise more than 10 percent of the cellular population is classified as a granulosa cell tumor. Spindle cell gonadal stromal tumors with only a minor granulosa cell component are best classified as a thecoma or fibroma with minor sex cord elements. (38) Some granulosa cell tumors are composed of spindle shaped cells. These differ from a thecoma in that the cells tend to be shorter and have the nuclear features of
granulosa cells, they have more abundant cytoplasm, and other patterns of granulosa cell tumor are often present. A reticulin stain can be helpful in evaluating such tumors, since in a granulosa cell tumor the reticulin fibers tend to surround groups of cells, while in a thecoma or fibroma the fibers surround individual tumor cells. Rare granulosa cell tumors contain heterologous mucinous epithelium or are composite tumors with mucinous elements. (39-43) A few granulosa cell tumors with focal hepatocellular differentiation have been reported. (44, 45) The hepatoid cells are difficult to differentiate from luteinized cells or Leydig cells in H&E stained slides; immunostains are necessary to make the distinction.

Unfortunately, it is difficult for pathologists to provide prognostic information; the pathologic findings correlate poorly with the clinical behavior, although some findings correlate to a degree with the outcome. (46) The prognosis is less favorable for large tumors more than 15 cm in diameter, for bilateral tumors, and for those that have ruptured or spread beyond the ovary (i.e., FIGO stage >IA). The stage is the single most powerful prognostic indicator, so it is important to provide adequate staging information in the pathology report. (16, 47, 48) Tumors with diffuse moderate or marked nuclear atypia or frequent mitotic figures (variably defined as greater than 2 or 4 mitotic figures per 10 high power fields) have been thought to be more likely to recur, but in recent studies neither the mitotic rate nor the proliferation rate as measured by the frequency of Ki-67 positive nuclei has correlated with the clinical outcome. (48-50) As mentioned above, there is no correlation between the microscopic pattern and the clinical outcome.

Small non-neoplastic granulosa cell proliferations that somewhat resemble small adult granulosa cell tumors are occasionally seen in the ovaries of women who are pregnant or postpartum. (51) These are small, multifocal, and confined to the antra of atretic follicles and they do not have the luteinized myxoid appearance of a granulosa cell tumor in pregnancy. (52) Strips and clusters of non-neoplastic granulosa cells are occasionally seen in vascular channels, perhaps being misplaced during surgery, pathologic evaluation or at ovulation. {McCluggage, 2004 #17426

**Immunohistochemistry and Molecular Pathology**

A missense somatic mutation that is characteristic of adult granulosa cell tumors has recently been identified in the FOXL2 gene (402C to G) located at 3q22.3. (53-55) This mutation is present in more than 90% of adult granulosa cell tumors, as well as in occasional sex cord-stromal tumors of other types, mainly thecomas, but so far not in other ovarian tumor types. (56, 57) Studies of tumors diagnosed as granulosa cell tumors that lack the mutation indicate that many, if not all, were other types of tumors misclassified as granulosa cell tumors. There is no correlation between the presence or absence of the mutation and immunostaining for FOXL2.

Immunohistochemical stains can be very helpful in the diagnosis of granulosa cell tumor. Nearly all granulosa cell tumors are vimentin positive. (58-60) Most granulosa cell tumors are keratin negative, but up to a third show focal or diffuse staining for keratin, particularly with antibodies directed against low molecular weight cytokeratins 8 and 18. (60) Dot-like or globoid perinuclear staining is particularly suggestive of a granulosa cell tumor, but extensive perinuclear or diffuse cytoplasmic staining can be seen. Absence of staining for epithelial membrane antigen (EMA) is an important characteristic of granulosa cell tumors and other sex cord-stromal tumors that helps to differentiate them from various types of epithelial tumors. (61, 62) There is positive staining for smooth muscle actin in most tumors, but granulosa cells generally do not stain for desmin. (62, 63) About 50 percent of granulosa cell tumors show positive nuclear or cytoplasmic
staining for S-100 protein.\(^{(62)}\) There is membrane staining for CD99 (MIC2 gene product) in about 70 percent of granulosa cell tumors.\(^{(64-67)}\)

There are a number of positive markers for granulosa cell tumor, all of which are also positive to varying degrees in other types of sex cord-stromal tumors.\(^{(67)}\) Most granulosa cell tumors show cytoplasmic staining for inhibin, although it is often patchy and variable in intensity.\(^{(68-76)}\) Most epithelial tumors are inhibin-negative, but focal or diffuse positive staining is occasionally detected. Calretinin is also an excellent marker for granulosa cell tumors, with staining in both the cytoplasm and the nuclei of the tumor cells. It too is positive in other types of sex cord-stromal tumors and in mesotheliomas and in mesothelial hyperplasia.\(^{(75, 77, 78)}\) Two newer immunostains, steroidogenic factor-1 (SF-1) and FOXL2, are nuclear stains that are almost invariably positive in granulosa cell tumors.\(^{(79)}\) FOXL2 staining is present in virtually all granulosa cell tumors irrespective of whether or not they have a FOXL2 mutation. FOXL2 and SF-1 stain most types of sex cord tumors, but FOXL2 tends to be negative in steroid cell tumors. Other immunostains that are typically positive in granulosa cell tumors are WT1,\(^{(67)}\) which shows nuclear staining, and CD56,\(^{(80, 81)}\) which shows strong membrane staining. These antibodies react with various other tumor types as well. Granulosa cell tumors can show cytoplasmic staining for CD10, but it tends to be weak and focal.\(^{(82)}\) A good limited immunohistochemical staining panel for the diagnosis of granulosa cell tumor could include inhibin and FOXL2 as positive markers and EMA, which should be negative. I also like to stain for cytokeratin, which, along with EMA picks up most carcinomas. SF-1 and calretinin are also useful at times. Vascular endothelial growth factor (VEGF) and its receptor can be demonstrated in many granulosa cell tumors with immunohistochemistry.\(^{(83)}\) Depending on how clinical therapy for granulosa cell tumors develops, this test could become relevant as clinicians are starting to treat some patients with recurrent granulosa cell tumors with the monoclonal antibody bevacizumab, and positive staining for these antigens might suggest a greater likelihood of a favorable response.\(^{(28)}\)
Probably many of you have noted that this case is not a typical granulosa cell tumor because a second, different histologic pattern is present. The appearance of the tumor varies from slide to slide. This is what the contributing pathologist had to say about the case:

**Microscopic Description:**
Sections of the left ovarian mass reveal a granulosa cell tumor with a variable histologic appearance. Approximately half of the sections examined exhibit a macrofollicular pattern typically seen with a juvenile granulosa cell tumor. The remaining sections exhibit histologic patterns ranging from microfollicular to trabecular to insular to solid areas of tumor growth. Areas of necrosis and degeneration are identified. In the most mitotically active regions of the tumor the mitotic rate is approximately 18 mitotic figures per 10 high-powered fields (block A6). Focal cytologic and nuclear atypia are also identified. The capsule appears to be intact histologically. All staging biopsies, lymph nodes, and omentum are free of tumor.

**Pathologist Comment:**
It is difficult to classify this granulosa cell tumor into either the juvenile or adult type. Approximately one-half of the tumor is composed of a macrofollicular pattern of growth which is typically seen within juvenile granulosa cell tumors. The remaining tumor exhibits a variety of histologic patterns typically seen in adult granulosa cell tumors. The presence of a large macrofollicular pattern, the increased rate of mitotic activity, and the size of this tumor are possibly suggestive of a more aggressive biological behavior. However, the behavior of stage I granulosa cell tumors cannot be accurately predicted on the basis of histologic appearance alone.

This is a granulosa cell tumor with a mixture of adult and juvenile granulosa cell tumor patterns. I have seen a few other examples. Their existence is surprising, since the adult and juvenile types of granulosa cell tumor are two different tumor types, not simply two grades of the same tumor. The FOXL2 mutation that is present in adult granulosa cell tumors is not present in the juvenile type. One occasionally sees mixtures of different patterns of sex cord stromal tumors in ovarian neoplasms, and this seems to be an example of such a case. Thus, the diagnosis is:

**Diagnosis: Granulosa cell tumor with a mixed pattern of adult granulosa cell tumor and juvenile granulosa cell tumor.**

**Juvenile Granulosa Cell Tumor**
Fewer than 5 percent of granulosa cell tumors occur in children and teenagers. Most of those that do have distinctive clinicopathologic features. (84, 85) Juvenile granulosa cell tumors are the most common sex cord-stromal tumors in children. (86) They also occur in the testes in young boys.

Although they are called juvenile granulosa cell tumors, this type of neoplasm can occur at any age, from infancy to old age. Most do occur in children. (84, 85) The average patient age is 15 years, but in a study limited to children in a pediatric tumor registry, the average age was only 7.1 years. (87)

Juvenile granulosa cell tumors often secrete estrogens secreted that cause isosexual precocious pseudopuberty. Premenarcheal girls often (50–75 percent) have breast development, growth of pubic and axillary hair, vaginal bleeding, and increased bone age and an estrogen effect can be seen in a vaginal smear. Older children and premenopausal women develop menstrual abnormalities, including amenorrhea. A third to half of all patients have only nonspecific symptoms such as abdominal distention, pain, or a palpable abdominal mass or they develop acute abdominal symptoms due to torsion or rupture of the tumor. An adnexal mass is palpable in more than 70% of patients. With rare exceptions, juvenile granulosa cell tumors are unilateral, and more than 95% are limited to the ovary (stage I). The prognosis appears to be
worse for patients with stage IC tumors, so it is important to collect peritoneal washings for cytologic evaluation and to pay close attention to the status of the capsule. There is an association between juvenile granulosa cell tumor and Ollier’s (enchondromatosis) and Mafucci’s (enchondromatosis and multiple subcutaneous hemangiomas) syndromes. (84, 88-91)

Juvenile granulosa cell tumor is typically encapsulated and confined to one ovary at diagnosis (stage IA) and is treated by unilateral salpingo-oophorectomy. (92) Most patients are young, so hysterectomy and bilateral salpingo-oophorectomy used only for the few patients who present with advanced disease, or for the occasional older patient with this type of tumor. Pelvic and abdominal lymph node metastases are uncommon and it does not appear to be necessary to dissect them for staging or treatment purposes. (21) The long-term survival is good, but patients whose tumors rupture, or who have positive peritoneal cytology or extraovarian tumor spread have a significant risk of recurrence. (84, 85, 93) If the tumor does recur, the recurrence is generally detected within 3 years. Some patients with advanced, persistent or recurrent disease respond to platinum-based combination chemotherapy. (94, 95) Inhibin and müllerian inhibitory substance can be used as tumor markers for the follow-up of patients with juvenile granulosa cell tumors.

**Gross Pathology**

Juvenile granulosa cell tumors vary greatly in size, from 2.5 to 30 cm in diameter; the average is 12 cm. Most tumors are partly solid and partly cystic, but they can be completely solid or mainly cystic. Solid areas are yellow or tan. Hemorrhage is frequent, but necrosis is uncommon.

**Microscopic Pathology**

The granulosa cells in juvenile granulosa cell tumors are large, polygonal to spindled in shape and they have a variable, but usually abundant, amount of amphophilic or pink cytoplasm. Focal or extensive luteinization is a typical finding. The tumor cells have large round hyperchromatic nuclei that lack grooves and may contain conspicuous nucleoli (Fig. 15-4). Cells with enlarged pleomorphic nuclei and multinucleated cells are common. Mitotic figures tend to be numerous and average around 6 per 10 hpf.

There is typically a mixture of cysts and solid areas in juvenile granulosa cell tumors. The solid areas have a lobulated or nodular appearance at low magnification. Macrofollicular, diffuse solid, and cystic growth patterns are characteristic of juvenile granulosa cell tumor. The macrofollicles tend
to vary considerably in size and they have irregular shapes (Fig. 15-3, irregular macrofollicles). Their lumens contain mucinous material, they are lined by one or more layers of granulosa cells, and they are sometimes surrounded by a rim of theca cells. The solid areas consist of sheets of granulosa cells with a variable admixture of spindle shaped thecal or fibroblastic stromal cells. Vague or well defined papillae lined by granulosa cells occasionally grow into cystic spaces. The typical growth patterns of adult granulosa cell tumors, such as the microfollicular and insular patterns are usually not present, but the granulosa cells grow in a trabecular or tubule-like pattern in some tumors.

**Immunohistochemistry and Molecular Pathology**

The FOXL2 (C402G) mutation that characterizes adult granulosa cell tumors is absent in juvenile granulosa cell tumors. (53, 96) These two tumors, both of which are derived from granulosa cells, thus likely have a different histogenesis.

The immunohistochemical features of juvenile granulosa cell tumor are similar to those of adult granulosa cell tumors, with a few differences. The tumor cells are vimentin positive and they stain for low molecular weight cytokeratin in a quarter to half of the cases. Sex cord stromal markers are usually positive. There is cytoplasmic staining for inhibin, nuclear and cytoplasmic staining for calretinin, nuclear staining for FOXL2 and steroidogenic factor-1 (SF-1) and membrane staining for CD99 and CD56. (79) Staining for CD99 tends to be more intense in juvenile granulosa cell tumors than in the adult type, and a greater percentage of tumors stain, so this marker is more useful for this type of granulosa cell tumor. EMA tends to be negative, but in contrast to what is seen in adult type granulosa tumors, which are always EMA negative, some juvenile granulosa cell tumors show focal weak staining for EMA. (97)

It is unclear whether there is any significance to the pattern of staining for FOXL2. Some authors report that more intense staining for FOXL2 correlates with more aggressive clinical behavior, (96) while others have reported exactly the opposite, namely that tumors that show loss of staining for FOXL2 are more aggressive. (98) Further study of this question is obviously indicated.

**Differential Diagnosis**

The differential diagnostic considerations obviously vary according to the type of granulosa cell tumor. For adult type tumors, the most significant differential diagnostic issue by far is carcinoma, either primary or metastatic. The differential diagnosis with thecoma is discussed above. Carcinoid tumors and lymphoma are other considerations. For juvenile granulosa cell tumor, the main differential diagnostic problems are yolk sac tumor and small cell carcinoma of the hypercalcemic type. Other considerations are listed in the tables.

*Primary and metastatic carcinomas* were not infrequently misdiagnosed as granulosa cell tumors in the past. This happens less often now, due to the wide availability of immunohistochemistry. The types of ovarian carcinomas that most often cause diagnostic problems are undifferentiated carcinoma, which grows in diffuse sheets mimicking the diffuse pattern of granulosa cell tumor, and endometrioid carcinoma, in which areas of spindle cell differentiation can mimic solid patterns of granulosa cell tumor, and which can form small tubular glands that mimic microfollicles. Among metastatic carcinomas, metastatic breast cancer is particularly likely to form microfollicle like glands that can cause confusion with a granulosa cell tumor. The degree of nuclear atypia and mitotic activity is generally much greater in a carcinoma than in a
granulosa cell tumor, so marked nuclear atypia or frequent mitotic figures should cause the pathologist to question a diagnosis of a granulosa cell tumor. Strong staining for keratin and EMA and lack of staining for granulosa cell tumor markers such as inhibin, calretinin and FOXL2 help establish the correct diagnosis.

Carcinoid tumors are composed of small to medium sized cells that grow in some of the same patterns as granulosa cell tumors, such as insular and trabecular patterns, so it is not surprising that they can be misdiagnosed as granulosa cell tumors. Carcinoid tumors are commonly associated with teratomas. The tumor cell nuclei frequently have distinctive coarse “salt and pepper” chromatin and the cytoplasm contains eosinophilic neuroendocrine granules that can be seen in H&E stained slides. These tumors show positive staining for markers of neuroendocrine granules such as chromogranin and synaptophysin, and they do not stain for granulosa cell markers.

Lymphoma can involve the ovary, usually as part of a systemic process, but occasional primary ovarian lymphomas occur. Lymphoma is somewhat similar to granulosa cell tumor at low magnification, in that both are tumors of small darkly stained cells. Lymphoma tends to infiltrate around ovarian structures, such as follicles, while granulosa cell tumor pushes them aside. At higher magnification, lymphoma cells have characteristic morphology which is different from that of a granulosa cell tumor. In problematic cases, immunostains readily resolve the differential diagnosis, and lymphoma cells mark for a variety of lymphoma markers and not for granulosa cell tumor markers. Most ovarian lymphomas are B-cell lymphomas and they show strong staining for CD20, as well as general markers like CD45 (LCA).

Juvenile granulosa cell tumor is most likely to be mistaken for a germ cell tumor or for small cell carcinoma of hypercalcemic type.

Small cell carcinoma of hypercalcemic type occurs in the same age range as juvenile granulosa cell tumor, and has some similar histologic features, including growth of tumor cells in diffuse sheets and the formation of irregular macrofollicles. The tumor cells tend to be smaller and darker than those in juvenile granulosa cell tumor, and the mitotic rate is often higher. Luteinized tumor cells do not occur, although luteinized stromal cells are occasionally present in the background. A large cell variant of small cell carcinoma has been described, but the tumor cells have a rhabdoid appearance with less cytoplasm than is present in the luteinized cells of juvenile granulosa cell tumor. Small cell carcinoma tumor cells are EMA positive and they do not stain for markers of juvenile granulosa cell tumor such as inhibin and, in very limited studies, FOXL2.
Germ cell tumors such as dysgerminoma and yolk sac tumor can have areas that resemble a juvenile granulosa cell tumor. Alveolar patterns in dysgerminoma mimic the irregular macrofollicles of a juvenile granulosa cell tumor. Areas of solid growth, labyrinthine growth, papillary growth and myxoid areas in yolk sac tumors mimic similar patterns that occur in juvenile granulosa cell tumor. The overall appearance of these tumors as seen in multiple slides is generally sufficiently distinctive to permit their differentiation from juvenile granulosa cell tumor, however. The nuclei of yolk sac tumor cells have a primitive, embryonal appearance that is not seen in juvenile granulosa cell tumors. Dysgerminoma is typically strongly positive for CD117, D2-40, OCT4 and SALL4, while yolk sac tumor shows staining for SALL4, glypican-3, and AFP, none of which are positive in juvenile granulosa cell tumor cells. The germ cell tumors lack staining for granulosa cell tumor markers. One thing to watch out for is the presence of luteinized stromal cells in the background of germ cell tumors; these often stain for sex cord-stromal tumor markers, but the actual germ cell tumor cells are negative. Another potential pitfall is the presence of hepatoid cells in granulosa cell tumors, which might stain for AFP and glypican-3, but the staining would be focal and limited to the hepatoid cells.

A variety of other small cell tumors can involve the ovaries either as primary or metastatic tumors and they can sometimes enter the differential diagnosis of granulosa cell tumor. (99) Apart from the tumor types discussed above, these include small cell carcinoma of neuroendocrine type, either primary or metastatic, desmoplastic small round cell tumor, PNET of central or peripheral type, endometrial stromal sarcoma, rhabdomyosarcoma, and neuroblastoma.

References

76. Shah VI, Freites NO, Maxwell P, McCluggage WG. Inhibin is more specific than calretinin as an immunohistochemical marker for differentiating sarcomatoid granulosa cell tumour of the ovary from other spindle cell neoplasms. J Clin Pathol. 2003;56(3):221-4.
Case 16
Steroid Cell Tumor

Clinical History: The patient was a 50 year old woman who presented with menometrorrhagia. During the course of her workup she had a pelvic ultrasound that revealed a slightly enlarged right ovary. It was thought to contain a 2.5 cm dermoid. She was followed, but the enlargement persisted and she elected to have a laparoscopic hysterectomy and BSO. At surgery, the ovaries appeared normal and the ovarian mass seen on ultrasound could not be visualized.

Gross Pathology: The right ovary measured 3.5 x 3.0 x 2.5 cm. The external surface was tan, lobular and intact. Cross sections revealed a 2.5 cm solid orange nodule.

Diagnosis: Steroid cell tumor, nos.

Steroid Cell Tumors

This category of ovarian tumors is a heterogeneous group of gonadal stromal tumors. (1) It includes the various types of Leydig cell tumors and the stromal luteoma, all of which are usually small and clinically benign. The other tumors in this category are steroid cell tumors, not otherwise specified (NOS), a group that includes small clinically benign tumors and larger pathologically and clinically malignant tumors.

Leydig cell tumor, hilar type
Leydig cell tumor, nonhilar type
Stromal Leydig cell tumor
Stromal luteoma
Steroid cell tumor, nos

Leydig Cell Tumor

Most ovarian Leydig cell tumors develop in the hilum, presumably from hilus cells. This is why hilar Leydig cell tumors were called “hilus cell tumors” in the past. Leydig cell tumors can also develop from the ovarian stroma outside the hilum where they are called non-hilar or stromal Leydig cell tumors depending on their morphology. (2-4) These tumors account for about 20% of steroid cell tumors.

Leydig cell tumors mainly occur in postmenopausal women. The average patient is in her upper 50s and almost all patients are more than 30 years old. (5) The usual clinical presentation is with hirsutism or signs of virilization such as acne, hair loss, deepening of the voice, development of a male body contour or hypertrophy of the clitoris. The serum testosterone concentration is elevated in virilized patients, but urinary 17-ketosteroids are generally within normal limits. Patients who are not virilized usually present with abnormal uterine bleeding. The tumor is an incidental finding at surgery for some other condition in a small percentage of cases. The endometrium is usually atrophic, but in a minority of patients peripheral conversion of testosterone to estrogen results in an abnormal endometrial pattern, including postmenopausal...
proliferative endometrium, hyperplasia or even adenocarcinoma. (6) Symptoms are often present for several years before the diagnosis is made. This is partly because Leydig cell tumors are usually small and difficult to localize, leading to consideration of other diagnoses, such as adrenal dysfunction. Non-palpable tumors can sometimes be detected by imaging studies. It is not uncommon for the clinician to have to measure hormone concentrations in blood obtained by selective catheterization of the ovarian veins to determine which ovary contains the tumor. (7-9) From a practical point of view Leydig cell tumors are benign and cured by surgery. (2, 5) Signs of virilization ordinarily regress following removal of the tumor.

**Gross Pathology**

Leydig cell tumors are unilateral small solid brown or yellow-brown tumors located in the hilum of the ovary, or, rarely, in the medulla or cortex. The average diameter has been reported to be between 2 and 3 cm, but tumors we have seen recently have tended to be even smaller, and often not visible until the ovary was sectioned.

Fig. 16-1 Polygonal cells, vesicular nuclei

**Microscopic Pathology**

Hilar Leydig cell tumors are circumscribed but not encapsulated. The tumor cells are typical Leydig cells as seen in the testis and around nerves in the hilum of the ovary. They are round or polygonal cells and have abundant granular eosinophilic cytoplasm (Fig. 16-1). Some cells contain yellow or brown lipochrome pigment. The tumor cells have round and uniform nuclei of medium size. The nuclei are vesicular or hyperchromatic and conspicuous nucleoli are often present. The tumor cells grow in diffuse sheets. There is a tendency for the tumor cells to be arranged in such a manner that their nuclei are clustered, leaving cytoplasmic nuclear free zones within the tumor. A peculiar eosinophilic fibrinoid change is sometimes present in the walls of blood vessels. (5) Crystalloids of Reinke are the characteristic marker of Leydig cells. These are intracytoplasmic eosinophilic hyaline rods with blunt or tapered ends. Intracytoplasmic eosinophilic hyaline globules, which are thought to be precursors of Reinke crystals, are sometimes easier to find. Unfortunately, Reinke crystalloids can only be identified in about 50 percent of hilar Leydig cell tumors, so they are an unreliable marker of this type of tumor, which is diagnosed based on the typical tumor cell cytology and the hilar location.

There are two types of non-hilar Leydig cell tumors, both of which are quite uncommon. Pure non-hilar Leydig cell tumors are circumscribed tumors that are grossly and microscopically identical to hilar Leydig cell tumors except for their location; they are usually centered in the medulla. (4) Stromal Leydig cell tumors are fibromas or thecomas that contain clusters, nests, or sheets of Leydig cells. (2, 3, 10) By definition, crystalloids of Reinke must be identified in the tumor cells in order to diagnose a non-hilar Leydig cell tumor. Since only 50 percent of hilar Leydig cell tumors contain crystalloids, it is obvious that many non-hilar Leydig cell tumors go unrecognized and are misdiagnosed as stromal luteomas or luteinized thecomas.
Stromal Luteoma

Stromal luteoma is a rare estrogen-secreting tumor that occurs mainly in postmenopausal women. (11) It accounts for about 20% of steroid cell tumors. The most common presentation is with abnormal uterine bleeding, and an endometrial biopsy often reveals a proliferative pattern or hyperplasia. Rare stromal luteomas are virilizing. A third of cases are incidental findings at operation or autopsy. The stromal luteoma is clinically benign.

Gross Pathology

Stromal luteoma is a small unilateral neoplasm; all reported examples have been less than 3cm in diameter, but they are often smaller and may not be detected until the ovary is cut open. The cut surface is gray, white, yellow, or brown.

Microscopic Pathology

The tumor is located in the ovarian stroma and is composed of luteinized stromal cells. The cells are polygonal, with ample granular eosinophilic cytoplasm and small round central nuclei. There is no nuclear atypia and mitotic figures are rare or absent. They are differentiated from a Leydig cell tumor by their non-hilar location and by the absence of cytoplasmic crystalloids of Reinke. The background ovary typically shows bilateral stromal hyperthecosis and, often, hilus cell hyperplasia. (11)

Steroid Cell Tumor, NOS

This category of ovarian tumors encompasses stromal neoplasms that cannot be more specifically classified, hence the designation “NOS” (not otherwise specified). Tumors of this type have also been called “lipid cell tumors” because many of them have clear foamy cytoplasm. (12) Most tumors are composed of a variable mixture of cells resembling Leydig cells, but lacking crystalloids of Reinke, and cells resembling adrenal cortical cells. Our case 16 falls into this category.

Steroid cell tumors occur over a wide age range, from 3 to 80 years. The average patient is a middle-aged woman about 45 years old, but these tumors occasionally occur in children or in the elderly. (13-15) Most steroid cell tumors secrete androgenic steroids in amounts sufficient to cause hirsutism or virilization. Serum testosterone concentrations and urinary 17-ketosteroids are elevated in virilized patients. Some tumors secrete renin and are associated with secondary polycythemia or hypertension. (16, 17) Rare steroid cell tumors secrete cortisol and cause Cushing’s syndrome. (18) Patients who do not present with hormonally mediated symptoms have abdominal distention or pain, menstrual irregularities, or postmenopausal bleeding. The tumor is an incidental finding in a few patients.

Most tumors are confined to the ovaries at diagnosis and bilateral tumors are rare (6 percent). (19) Metastases, usually to the peritoneum, are present at the time of diagnosis in 10–20% of cases. (12, 19) Young patients with stage IA neoplasms can be treated by salpingo-oophorectomy. Older patients and those with advanced tumors are generally treated by hysterectomy and bilateral salpingo-oophorectomy. Hirsutism and signs of virilization regress after removal of the tumor. A major difference from the other tumors in the group (Leydig cell tumors, stromal luteoma) is that a significant proportion of steroid cell tumors, 25–43%, including a majority of those that cause Cushing’s syndrome, are clinically malignant. (12, 18, 19) Recurrences are generally discovered within the first few years after treatment, but about 20% are detected after more than 5 years.
Gross Pathology
Steroid cell tumors are solid and range from less than 1 cm to more than 20 cm in diameter; the average is about 7 cm. The cut surface is tan, yellow, or orange, and about 25% have areas of hemorrhage and necrosis. A few parovarian steroid cell tumors, possibly originating in ectopic ovarian tissue or perhaps in adrenal rests, have been reported. (20, 21)

Microscopic Pathology
A mixture of Leydig-like and adrenal cortical-like cells is generally present, although one of these may predominate. The Leydig-like cells are round or polygonal and have abundant, sometimes vacuolated eosinophilic cytoplasm. The nucleus is round, centrally located with a small nucleolus. Crystalloids of Reinke are never identified. The adrenal cortical-like cells are also round or polygonal and have abundant pale or clear vacuolated cytoplasm. The nucleus is vesicular and may contain a small to medium sized but conspicuous nucleolus. Fat stains are positive in adrenal-type cells. In most cases, mitotic figures are infrequent and nuclear atypia is absent or modest. Our case is an interesting one that at first glance appears to contain a typical mixture of Leydig-like and adrenal cortical-like cells, with the latter predominating. The cells with eosinophilic cytoplasm, however, are the ones with the largest nuclei and the ones that show mitotic activity, and they actually more resemble eosinophilic adrenal cortical cells than Leydig cells.

Pathologic features that have been present in clinically malignant steroid cell tumors include large size, hemorrhage or necrosis, 2 or more mitotic figures/10 high power fields, and moderate or marked nuclear atypia. (19)

Immunohistochemistry of Steroid Cell Tumors
All of the tumors in this group have similar immunophenotypes. Steroid cell tumors differ from other types of sex cord-stromal tumors in that they are mostly FOXL2 and WT1 negative. (22, 23) On the other hand, they typically show strong granular cytoplasmic staining for melan-A or for the related marker MART-1. (23-25) There is strong cytoplasmic staining for inhibin, cytoplasmic and nuclear staining for calretinin and nuclear staining for steroidogenic factor-1. (23, 25) CD56 stains tumor cell membranes. In one study there was strong membrane staining for CD99 in cells with eosinophilic cytoplasm, but cells with clear vacuolated cytoplasm were generally negative, (24) while in other studies only a minority of steroid cell tumors stained for CD99. (23, 26) The tumor cells are vimentin positive. There is focal staining for cytokeratin in 30–50% of cases, most often in cells with clear vacuolated cytoplasm, and often in a
perinuclear globoid or dot-like pattern. (24, 27) Other reactions that have been reported include positive staining for androgen receptors in about two thirds of cases, (24) positive staining for smooth muscle actin in a third of cases, (27) and negative staining for EMA. The best combination of stains to confirm the diagnosis is inhibin, melan-A and steroidogenic factor-1; lack of staining for EMA helps to exclude a clear cell carcinoma.

**Differential Diagnosis**

Steroid cell tumors are generally not difficult to diagnose, although their subclassification is problematic in some cases due to the difficulty of identifying crystalloids of Reinke.

*Nodular hyperplasia* of luteinized stromal cells or hilar cells can occasionally raise the question of a Leydig cell tumor or a stromal luteoma, but hyperplastic nodules are microscopic and usually multifocal.

*Luteoma of pregnancy* is composed of luteinized stroma cells and its appearance overlaps with that of the tumors in this group. However, it occurs in a very particular clinical setting, namely in a young woman who is pregnant or who has just delivered. The luteoma of pregnancy is typically associated with a theca lutein cyst, and there are often multiple nodules in the ovary, and in the contralateral ovary as well. No crystalloids of Reinke are present.

*Clear or oxyphilic cell tumors* of various types occur in the ovary, either as primary or metastatic neoplasms. These include clear cell carcinoma, variants of endometrioid carcinoma, hepatoid tumors of various types, and metastatic clear cell renal cell carcinoma. Most of these have an obviously more malignant appearance than would be anticipated in a steroid cell tumor, with marked atypia or numerous mitotic figures. They tend to be strongly positive for keratin and EMA, and they do not stain for steroid cell tumor markers.

*Luteinized granulosa cell tumor* may enter the differential diagnosis. This tumor is rarely completely luteinized, and areas of more typical granulosa cell differentiation are generally present. Granulosa cell tumor is more likely to be estrinizing. Immunohistochemistry may be helpful, as most granulosa cell tumors, including luteinized ones, are FOXL2 positive and this stain is often negative in steroid cell tumors.

**References**