Germ Cell Tumors of the Ovary and Dysgenetic Gonads

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Abstract

Malignant ovarian germ cell tumors account for less than 5 % of ovarian cancers. However, since these tumors primarily involve women during the reproductive years, making fertility-conserving treatment an important matter, they are highly significant. Germ cell neoplasms are also of interest because some of the processes involved in their genesis relate to early embryonic development, reproduction, and determination of an individual's sex – all fascinating and important matters.

Introduction

Malignant ovarian germ cell tumors account for less than 5 % of ovarian cancers [1]. However, since these tumors primarily involve women during the reproductive years, making fertility-conserving treatment an important matter, they are highly significant. Germ cell neoplasms are also of interest because some of the processes involved in their genesis relate to early embryonic development, reproduction, and determination of an individual's sex – all fascinating and important matters.

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Gonadal Development

Though it is considered a matter of common sense and beyond argument that we mammals are easily categorized into either male or female sex, in fact, determination of biological sex is extremely complex. Basically there is no one biological character at the genotypic level or at the phenotypic level that can be said to be an exclusive determinant of either the male or the female sex. In basic biological terms it is not entirely clear why the condition known as dioecy (in which the male and female gametes are produced by different individuals) should have evolved. The basic biological parameter distinguishing male from female is considered to be anisogamy – the different size of the male and female gametes; usually a male individual produces large numbers of small gametes, whereas the female produces fewer but larger gametes. Even this is not universally so. For example, that much observed animal, *Drosophila*,

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famously produces giant sperm, which, on the basis of length, might be considered larger than the corresponding ovum [2].

Early in the development of the embryo, there is differentiation between the trophoblast and the inner cell mass resulting in development of the blastocyst and this occurs before implantation [3]. The cells of the inner cell mass are at this stage pluripotent and are embryonic stem cells (ESC). They express a gene that is known to be crucial for early embryonic development – the octomer-binding transcription factor 3/4 known variously as POU5F1 or OCT3/4 [3]. The gene product of this has proved incredibly useful at a practical level as a superb nuclear marker for seminoma/germinoma and embryonal carcinoma.

Though germ cells follow a sex-specific pathway, this is not dependent on their karyotype, but on the gonadal environment [4]. Though genetic sex in humans (if there actually can be considered to be such an entity) is established at the point of conception, depending on whether a Y-chromosome-bearing or an X-chromosome-bearing sperm fertilizes the ovum (which is always X-bearing), this is not all there is to sex development.

In their early development, the cells that are to become germ cells and which, at this stage, are referred to as primordial germ cells (PGCs) originate in the yolk sac from ESC [5] and migrate through the hindgut, controlled by the stem cell factor (SCF-)-c-KIT signaling system, c-KIT being the receptor for SCF expressed on the PGC [5]. c-KIT belongs to one of the families of tyrosine kinase receptors, which also includes platelet-derived growth factor receptor (PDGFR) and macrophage-colony-stimulating receptor (M-CSFR). The SCF-KIT pathway regulates the differentiation of melanocytes, red blood cells, mast cells, and interstitial cells of Cajal in the intestines, in addition to germ cells [6].

The primordial germ cells proliferate greatly as they migrate. Factors such as SOX17 are also crucial [7]. The PGC can be initially recognized at 5–6 weeks' gestation in the human embryo, even before they reach the gonads, when they are characterized by several markers such as alkaline phosphatase, VASA (a germ cell-specific RNA binding protein), c-KIT, and OCT3/4 [7]. OCT3/4 seems to maintain the pluripotent state in PGC and gonocytes (see paragraph below) and appears to have an anti-apoptotic function [5]. In the mouse, PGCs in the ovary and extra gonadal sites enter meiosis, but this is inhibited in the testis [3]. The extragonadal PGC usually die by BAX-dependent apoptosis [3].

Once in the genital ridge, where the gonad will develop, they are referred to as gonocytes [7], and at this point they can develop along either male or female lines, depending on how the environment around them develops. As mentioned above, gonocytes still express OCT3/4 [8]. Like all somatic cells, PGC and gonocytes have a *biparental* pattern of genomic imprinting. This is the phenomenon by which the maternal and paternal sets of chromosomes have different functions, due to parental-specific epigenetic modification [3]. At some point they lose their original biparental pattern of genomic imprinting completely; this must take place to allow proper development of gender-specific germ cell lineage [7].

In vertebrates, the gonad initially arises as a bipotential primordium that can develop into either ovary or testis; initially the cells are able to accept either pathway of differentiation because of balanced signaling and transcription networks. At the point at which the gonocytes arrive, the gonad is at the indifferent, bipotential stage. It is believed that the balanced antagonism of "male" factors (*SOX9*, *SRY*-box containing gene 9) and "female" factors (*Rspol*, R-spondin homologue) initially keeps the gonad in a bipotential state [4].

Differentiation into either sex occurs when either the testicular or the ovarian pathway is instigated and the other is suppressed [9]. This is a complex and dynamic process, as much of the genome is under transcription at this stage (indeed, it appears that half the genome is active) [9] and many genes are expressed in a sexually dimorphic way. While approximately 30 genes or so that seem to have a part in this process have already been identified, more than half of the many problems that arise in the development of the human gonads, known collectively as disorders of sex development (DSD), do not seem to

involve any of them, suggesting that there may be many other genes and processes involved in gonadal development that are as yet undiscovered [9]. One of the important genes involved in gonadal and renal development is the WT1 (Wilms' tumor 1) gene. This is expressed in the urogenital ridge during a very early phase of development [10].

There is evidence that many of the processes involved depend on the functioning of *SOX9*. Twenty years ago or so, the *SRY* (sex-determining region of the Y chromosome) was discovered; this is initially expressed at low levels in the testis, but when there is sufficient SRY protein present, this leads to expression of the transcription factor, *SOX9*. SOX9 expression occurs around week 7 of gestation [5]. This upregulation results in the formation of Sertoli cells and subsequently to (in the normal state) the development of the phenotypic male sex [7]. The actual expression of the male sex depends on the function of Leydig cells and testosterone production.

Initially, the Sertoli cells and gonocytes form cord-like structures and then seminiferous tubules. Initially, the gonocytes lie in the center of these and still have the markers characteristic of PGC and gonocytes [5]. However, they start to migrate to the periphery of the tubule, and when they reach the basal lamina, they mature to the stage of pre-spermatogonia and markers such as OCT3/4, PLAP, and c-KIT are lost, to the extent that there is almost no expression of OCT3/4 in the normal male neonate and none at all by the age of 4 months [5]. As will be discussed later in the section on assessment of dysgenetic gonads, in this situation, markers such as OCT3/4 persist for much longer.

Though, for many years, it was thought that ovarian development was a passive process that occurred in the absence of testicular development, in fact it is now thought that both gonads require dynamic contributions from complex networks of transcription factors in order to develop. While in the absence of functional *SRY* the stromal cells do normally develop into granulosa cells [7], ovarian development also requires the activation of several genes – including WNT4 and FOXL2 [8].

In the gonad, depending on the microenvironment, the gonocytes will differentiate into either oogonia or pre-spermatogonia. In the ovary, germ cells enter prophase of meiosis I, and in the testis they then attain a resting phase as spermatogonia during childhood. At puberty, meiosis commences in the testis and the spermatogonia differentiate into spermatocytes [4].

Germ Cell Tumors

The Heterogeneity of Germ Cell Tumors

It is well known that human germ cell neoplasms form a heterogeneous group and occur at several well-defined sites, including the gonads, both ovary and testis (which account for 90 % of them [11]) and other sites along the midline of the body (retroperitoneal, mediastinal, pineal/hypothalamic region) [12].

Though there are analogies between the germ cell tumors of the ovary and testis, there are marked differences between the two sites. One of the most obvious differences is the high frequency of mature cystic teratomas in the ovary compared to the testis (indeed 95 % of germ cell neoplasms in the ovary are teratomas [13]), whereas the frequency of some forms of malignant germ cell tumors, such as embryonal carcinoma, is much higher in the testis than in the ovary. Other sites also have peculiar features; the mediastinum, for example, is a site that is particularly associated with those with Klinefelter syndrome. Teratomas at this site have a strong association with hematological malignancy [14]

Germ cell tumors arise from embryonic germ cells that fail to fully differentiate and which then may undergo malignant transformation. The clinical course depends on many factors such as sex, age, and anatomical site. Many of the histological types of germ cell tumor, described in detail below in relation to the ovary, can occur at various sites, and, in many cases, their behavior as benign or malignant is similar at the various sites. Yolk-sac tumors (YSTs) are an interesting group that have a large range of histological appearances

because of their origin from the primary yolk sac of the embryo at the time of implantation [15]. This gives rise to many of the endodermal structures of the body, both foregut (the lung, liver, thyroid, stomach) and hindgut (the intestine and bladder epithelium) [15]. It is also the first organ of hemopoiesis [15] and the site of production of the major protein of embryonic life, alpha-fetoprotein (AFP) [15]. However, they have very variable biological behavior at the various sites at which they occur.

Though the testis has been the center of much of the research and classification of germ cell tumors (because of the relative frequency of malignant tumors at this site), there are close analogies with those germ cell tumors at other sites, including the ovary. This discussion will be limited almost entirely to ovarian germ cell tumors.

Types of Ovarian Germ Cell Tumor

The most recent World Health Organization classification of ovarian germ cell tumors [16] includes three basic categories:

- Teratomas (mature and immature)
- Primitive germ cell tumors (which includes tumors such as dysgerminoma, yolk-sac tumor, embryonal carcinoma, and non-gestational choriocarcinoma)
- Monodermal teratomas and somatic-type tumors associated with teratomas

This is a generally useful way to categorize ovarian germ cell tumors. The third category is particularly helpful as it includes thyroid-type tumors, primary carcinoid tumors, and the diverse group of tumors previously considered as malignant transformation of mature cystic teratoma. Somatic malignancy can arise in association with immature teratoma and is not limited to mature tumors [17], a matter recognized by this classification.

Ovarian Teratomas

Teratomas are common in the ovary and occur evenly throughout life. Overall, they account for 95 % of ovarian germ cell neoplasms, the majority of these occurring between the ages of 20 and 40 years [13]. Ovarian teratomas are most commonly benign in all age groups. In ovarian teratomas an important determinant of malignant behavior is the presence of immature elements, certainly in postpubertal individuals (see below). However, age may also have some effect on the prognosis of ovarian teratomas, as mentioned below.

Mature Cystic Teratoma (MCT)

Clinical Features

These are the most common germ cell tumors of the ovary, comprising 10–20 % of all ovarian tumors [18]. They occur over a broad age range, from infancy onward and throughout life, even occurring in the ninth decade [19], though with the greatest incidence during the reproductive years [13], when 80 % or so occur. They are the most frequently occurring ovarian neoplasm under 15 years of age, where they account for two thirds of all ovarian tumors [20]. Mature cystic teratoma is the only ovarian germ cell neoplasm to occur with any frequency at all in the first years of childhood; other germ cell tumors tend to occur from mid-childhood onward, though are much less frequent in children than in postpubertal women.

Most commonly they are asymptomatic and where there are symptoms, these tend to be non-specific and related to the presence of an abdominal space-occupying lesion. However, they may present with torsion (3.5 % in one large series [19]), which is more common in tumors measuring more than 10 cm in diameter. In rare cases, the tumor can leak into the adjacent tissues and lead to the presence of a mass resulting from the resulting reactive process in lymph nodes and fibroadipose tissue [19]. Teratomas may also present with a paraneoplastic autoimmune encephalitis associated with antibodies to receptor proteins [21]. This is discussed further below.

Macroscopic Appearance

The pathology is familiar; most tumors are predominantly cystic. There may be multiple cysts, but there is usually one large cyst which contains greasy material and hair, often with a mural nodule protruding into it. Well-formed teeth may form part of the tumor (Fig. 14.1). In one large series [19], half the tumors were between 5 and

10 cm in diameter with the mean maximum dimension being 6.4 cm, though with one (asymptomatic) tumor weighing over 7 kg and with a maximum dimension of 37 cm.

It is not uncommon for mature cystic teratomas to be bilateral; in the same large series, 10.8 % of mature cystic teratomas involved both ovaries [19]. This fact may be clinically significant; one study demonstrated that bilateral and multiple mature cystic tumors are associated with the development of future germ cell tumors, including germ cell malignancies, more frequently than single and unilateral tumors [22].

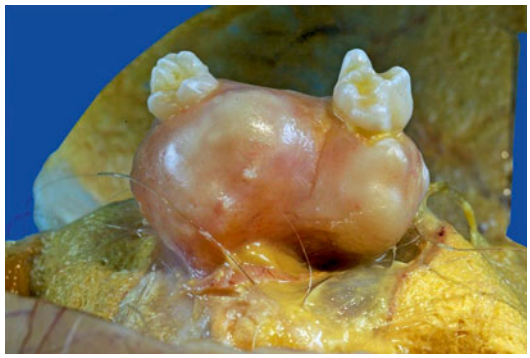

Fig. 14.1 Mature cystic teratoma, opened to show lining and the presence of teeth (Photo provided by Dr. Helen Stringfellow)

Microscopic Appearance

These tumors have a wall containing multiple mature tissues from ectoderm, mesoderm, and endodermal cell lines. The mature tissues represented frequently include skin and appendages (Fig. 14.2), choroid plexus, glial tissue (Fig. 14.3), thyroid tissue, bone, cartilage, teeth, and hair. More unusual constituents include pituitary and even prostate. The constituent tissues are usually well organized and lack mitotic activity.

Epidermoid Cyst

Some tumors that consist entirely of cysts lined by squamous epithelium are seen occasionally within the ovary, usually referred to as epidermoid cysts. These appear to be heterogeneous in origin and may not necessarily be teratomas, at least in all cases [23]. They are usually smaller than mature cystic teratomas and have a tendency to affect an older age group [23, 24]. They appear to be benign [24].

Prognosis

Most MCTs are treated and cured by excision of the cyst or the ovary. However, recurrence following excision is recorded [22]. Overall approximately 4 % of mature cystic teratomas recur, according to one large study [25]. Tumors that are bilateral, those involving women under

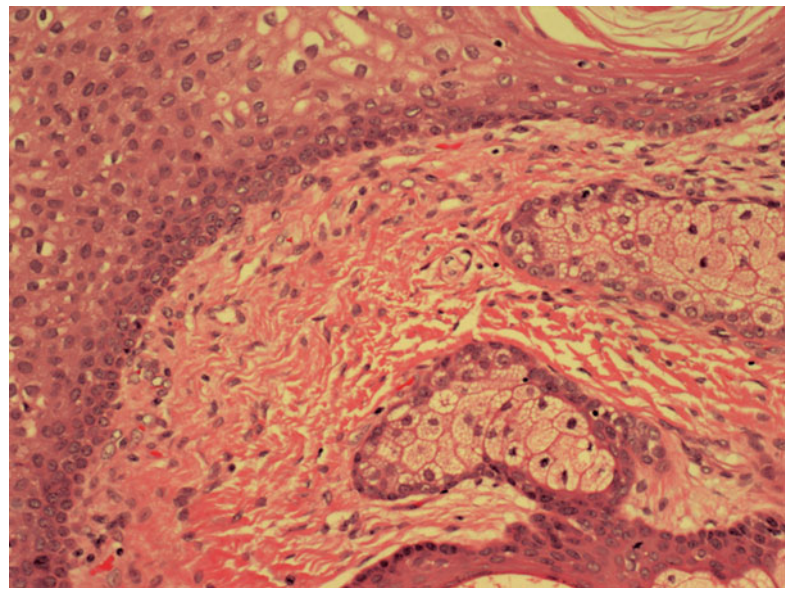

Fig. 14.2 Mature cystic teratoma – epidermal appendages, H&E ×200

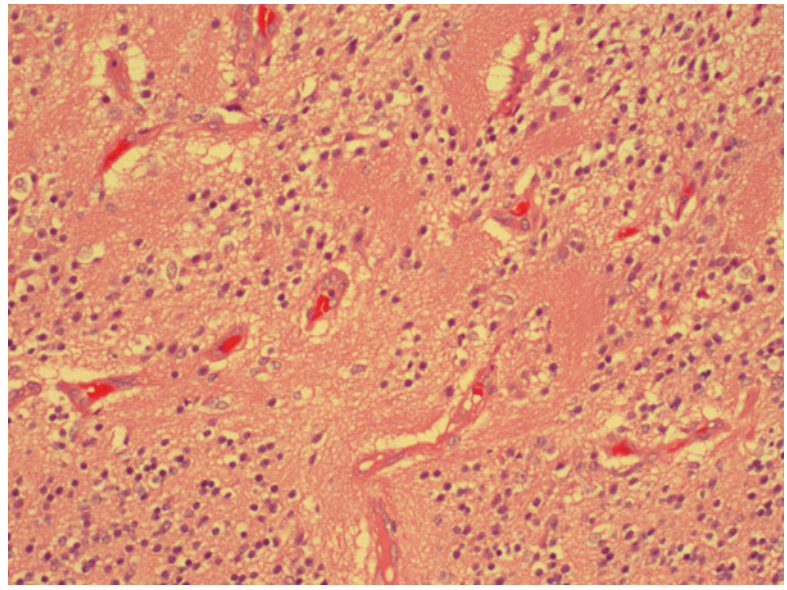

Fig. 14.3 Mature cystic teratoma – glial tissue, H&E ×200

30 years, and those with a diameter greater than 8 cm are more likely to recur [25]. One study (involving small numbers only) found that there are a small number of women who subsequently develop a malignant germ cell tumor following excision of MCT [22].

Small Foci of Immature Neural Tissue

Some mature cystic teratomas contain microscopic foci of immature neural tissue in the cyst wall. It can be difficult to distinguish these from some mature neural tissues, such as cerebellar cortex, which has a mature but small-celled internal granular layer (as shown in Fig. 14.6). However, sometimes small areas of truly immature neural tissue (which fulfill the criteria for this discussed in the section on immature teratoma) are found within the wall of an otherwise typical mature cystic teratoma (Fig. 14.4). From the relatively small number of cases considered in the literature, it seems that whereas bilateral or multiple dermoid cysts are associated with a higher recurrence risk and a greater chance of development of a subsequent immature teratoma, one or more small microscopic foci of immature neuroepithelium within the initial dermoid cyst do *not* appear to increase this risk [26]. The implication is, therefore, that these are unlikely to be of significance.

Cytogenetics

Mature cystic teratomas are diploid and have a 46,XX karyotype. They are usually considered to originate from germ cells following the first meiotic division, though this may be more complex and there may be other points of origin [27].

Other Forms of Mature Teratoma

Mature Solid Teratoma

This tumor affects the same age group as immature teratoma [17], discussed below, and therefore differs from MCT, which has a much wider age distribution. It is less common than its immature counterpart; only 10 % of solid teratomas are fully mature [17]. Macroscopically, these tumors are completely distinct from MCT – the familiar large cyst containing greasy material and hair is not seen and the appearance on naked eye examination can be indistinguishable from immature teratoma. Diagnosis depends entirely on thorough sampling – there are no immature tissues (particularly neuroepithelium) in a mature solid teratoma. Clinical and laboratory assessment is also important; for example, raised serum alpha-fetoprotein in a young women would warrant a careful search for primitive elements, such as yolk-sac tumor.

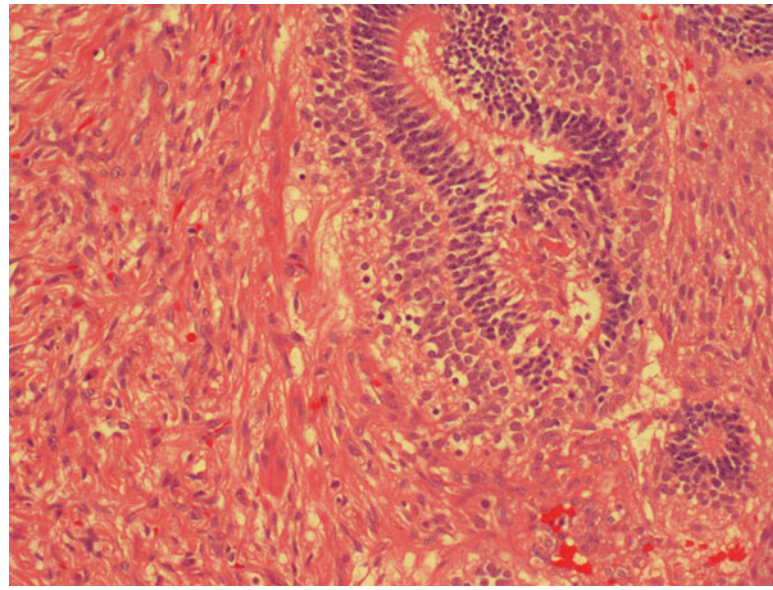

Fig. 14.4 Mature cystic teratoma – microscopic focus of immature neuroepithelium – H&E ×200

Teratomas Containing Highly Organized Mature Tissues

Sometimes, a teratoma can be so highly organized that it is difficult to distinguish the neoplasm from fetus in fetu [28], a malformation that does not usually involve the ovary [17].

Immature Teratoma

Clinical Features

Immature teratoma of the ovary is an uncommon tumor, comprising only 1 % of ovarian neoplasms and 20 % of all malignant ovarian germ cell tumors [29]. In one early but large study, the age of those involved ranged from 14 months to 40 years, with a median of 19 years [30]. Most were post-menarcheal, with less than 10 % known to be premenarcheal [30].

Presentation was for nonspecific reasons, abdominal mass, localized tenderness, abnormal bleeding or acute abdomen, and dyspareunia. There were no hormonal symptoms, though a minority had fever and leukocytosis [30]. As described above, there is an association with previous or synchronous MCT [13, 22, 26, 30].

Macroscopic Features

Immature teratoma is usually unilateral, though cases with the primary in one ovary and metastases to the contralateral ovary are recorded [30]. In one series tumors ranged between 7 and 35 cm in diameter [30]. Cysts were seen on slicing the tumor, though these could be very small, only a few millimeters in diameter, and could be distributed throughout the tumor. Some tumors did include larger cysts. The solid areas were variable, including "encephaloid" areas, hemorrhagic areas, or firm or gritty areas. Hair and teeth may be present [30], but the macroscopic appearance is distinct from the familiar MCT.

Microscopic Appearance

Immature teratoma contains embryonic tissue, though more mature tissues are also present, including many fully mature adult tissues. The immature elements are predominantly neuroepithelial, though endodermal and mesodermal elements may also be present (Figs. 14.5 and 14.6) They correspond to tissues present in the embryo 2–8 weeks following fertilization [17].

The appearance does vary with the grading of the tumor, described below. Grade 1 tumors tend to consist largely of mature tissue, often with immature mesenchyme, tooth anlage, and immature cartilage [30]. Grade 2 tumors often have a high proportion of immature tissue, whereas grade 3 tumors tend to include almost all immature tissues with a very cellular immature mesenchymal stroma. Hemorrhage and necrosis were most common in grades 2 and 3 [30].

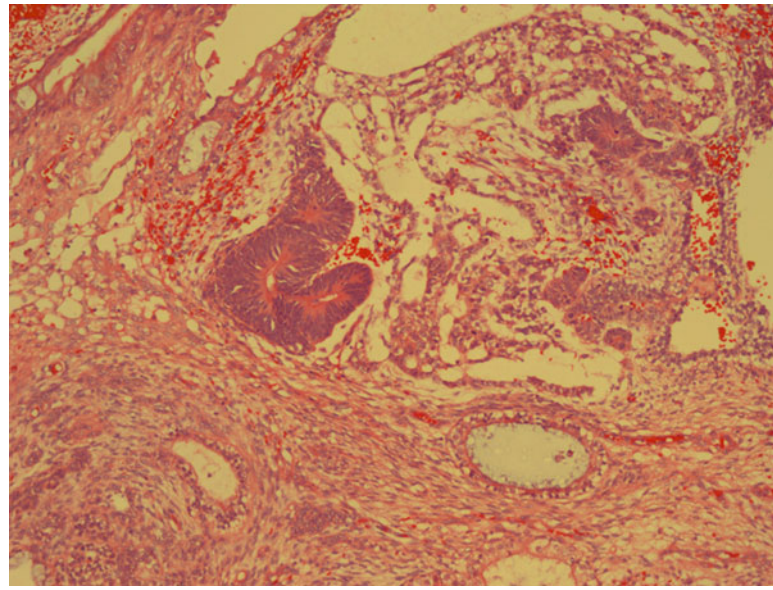

Fig. 14.5 Immature teratoma. Immature mesenchymal and endodermal elements, including a focus of immature neuroepithelium – H&E ×100

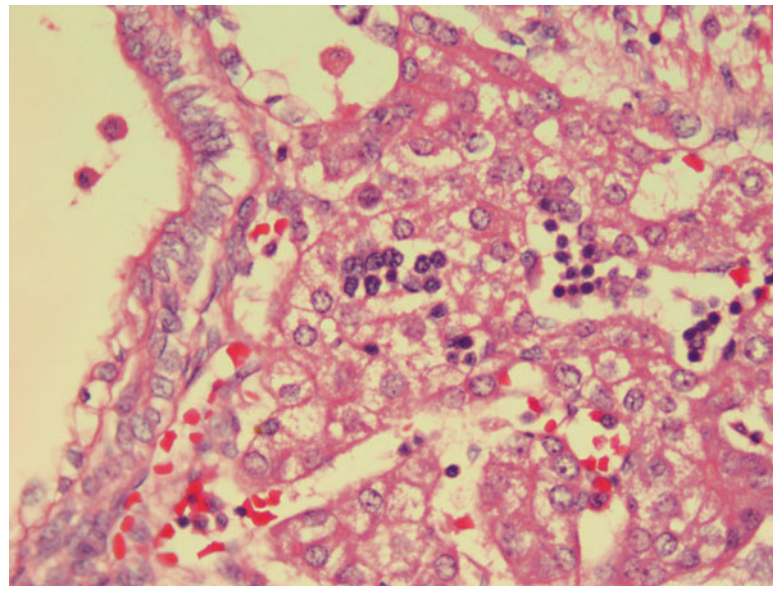

Fig. 14.6 Immature teratoma. Area of hepatic differentiation. Hemopoiesis also present. H&E ×400

Grading

The proportion of immature elements in an ovarian teratoma is important in grading and prognosis, at least in adults, and this has long been recognized. Thurlbeck and Scully first recognized that the amount of rosette-forming immature neural tissue was related to prognosis in immature teratoma [31]. To grade a tumor, it is necessary to examine the slide that includes the greatest proportion of immature tissues.

The relevant immature elements can be difficult to identify. They are almost always neural. Cellular differentiated elements (parts of the brain such as the cerebellar granular layer) (see Fig. 14.7) can be particularly difficult to distinguish from immature neuroepithelium, but the latter must have an embryonal appearance and usually show mitotic and apoptotic activity (see Fig. 14.8) [13]. The grading system is as follows:

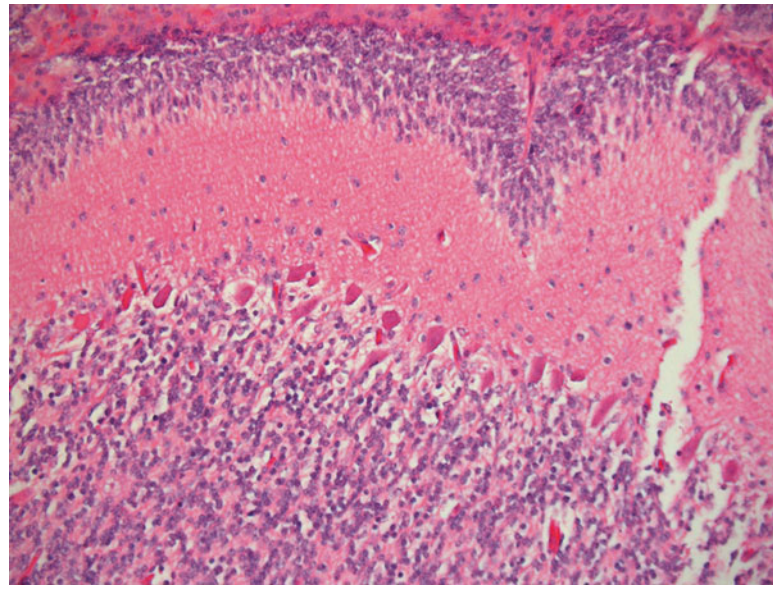

Fig. 14.7 Immature teratoma – cerebellar cortex (of infantile type) – this is not embryonal tissue, but differentiated tissue. The small granular cells may be deceptive, but do not show the mitoses and apoptosis H&E ×200

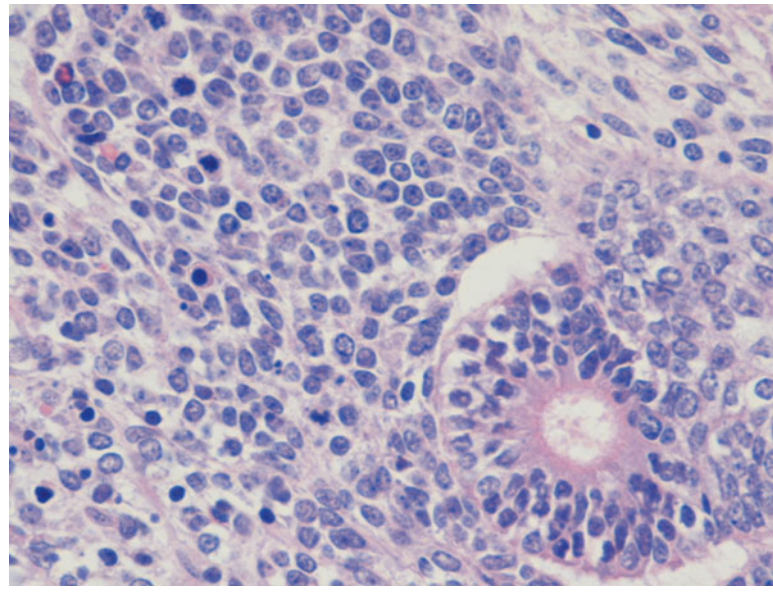

Fig. 14.8 Immature neuroepithelium in an immature teratoma. H&E ×400 (Photo provided by Dr Helen Stringfellow)

Grade 1 – Tumors with rare foci of immature neuroepithelium only – less than one low power (×4) field in any slide. In adult women, these tumors are associated with a survival of at least 95 % [13].

Grade 2 – Tumors with immature neuroepithelial elements that occupy 1–3 low power (×4) fields in the slide with the greatest proportion of immature tissue.

Grade 3 – Tumors with large amounts of immature neuroepithelium occupying more than 3 low power (×4) fields [29].

This has proved an efficacious way of classifying tumors, with grades 2 and 3 being regarded as high grade and requiring chemotherapy.

Prognosis

In postpubertal individuals, immature teratoma is a naturally aggressive tumor; prior to the modern chemotherapy era, the overall survival rate of the most immature tumors (highest-grade tumors) was only 30 % [29]. This has improved considerably following the advent of modern chemotherapy.

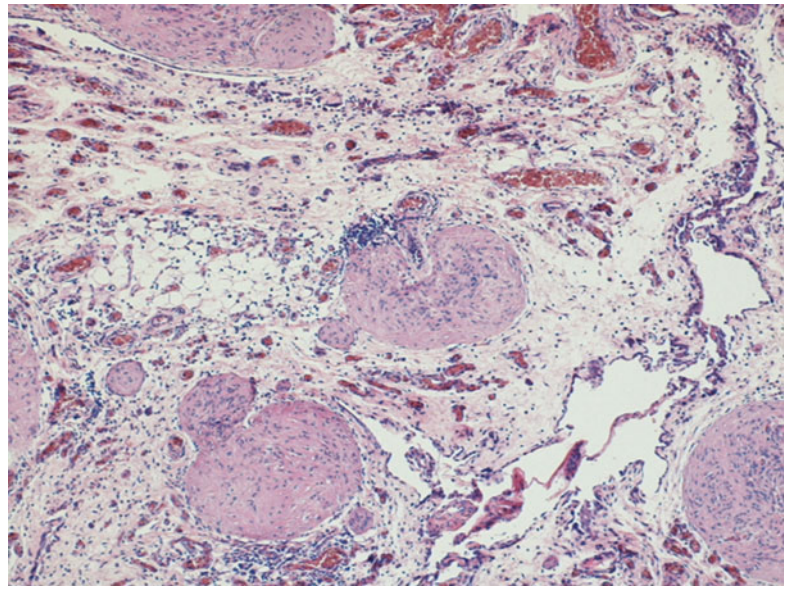

Fig. 14.9 Peritoneal glial nodule – gliomatosis. H&E ×100 (Photo provided by Dr Helen Stringfellow)

With modern treatment survival is reduced to around 85 % for grades 2 and 3. However, like many histopathological grading systems, there are problems in reaching a grade in some cases.

The marker OCT3/4 might be of assistance; it does appear to be present specifically in the nuclei of the most immature neuroepithelium and has been shown, in at least one study, to be expressed focally in the immature neuroepithelium of the high-grade teratoma (most commonly in grade 3 tumors), but not in the small foci of immature neuroepithelium of grade 1 tumors [29]. This suggests that it might be a useful marker in this context, though experience is, as yet, limited. As OCT3/4 is thought to have a key role in maintaining pluripotency and self-renewal and as it is observed in ESC and PGC, its expression in the immature tissue may be related to the pathobiology of the tumor [29].

In children, grading may not be of major relevance to prognosis as clinical studies have demonstrated an excellent outcome with surgery and follow-up only and with chemotherapy for relatively rare early relapse rather than universally [32].

Gliomatosis Peritonei

Immature ovarian teratomas are associated with gliomatosis peritonei (Figs. 14.9 and 14.10), nodules showing glial differentiation on the peritoneum. This is a favorable prognostic feature if fully mature. It has been demonstrated that this phenomenon is the result of teratoma-induced differentiation of submesothelial cells [33].

Growing Teratoma Syndrome (GTS)

This is the condition whereby metastatic masses of immature teratoma grow following or during treatment but contain only mature elements [34]. Surgical resection is the standard treatment [35]. Complications include local problems such as obstruction of intestine or ureter [36]. Malignant transformation of the residual tumor into sarcoma, carcinoma, or primitive neuroectodermal tumor or even carcinoid tumor is also recorded [36]. This appears to be rare; it occurred in 3 % of cases of growing teratoma syndrome in one series [36].

Monodermal Teratomas (and Teratomas Containing a High Proportion of One Tumor Type)

Monodermal teratoma term is used when there is only one tissue type identified within the tumor. Under the third WHO classification, these tumors are now regarded as *monodermal teratoma and somatic-type tumors associated with biphasic and triphasic teratoma* [16, 17] so that teratomas composed of a high proportion of these elements

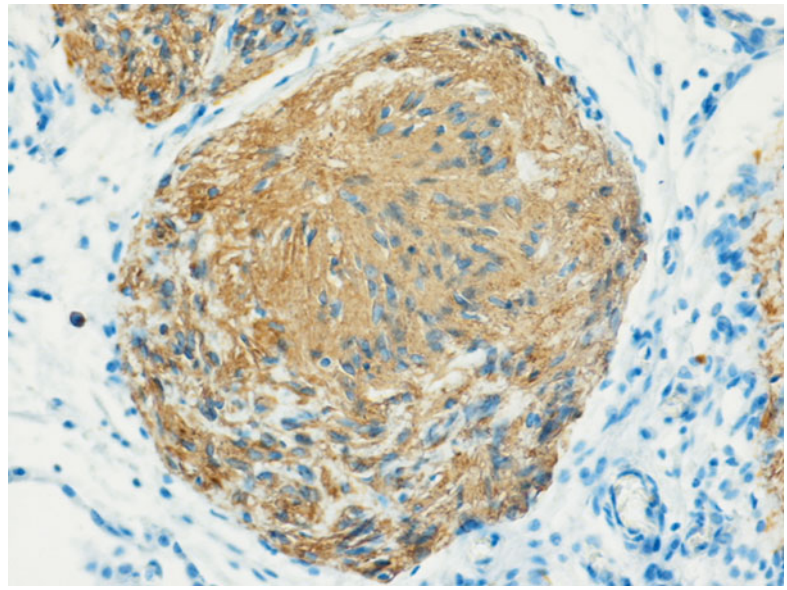

Fig. 14.10 Peritoneal glial nodule – gliomatosis. GFAP ×200 (Photo provided by Dr Helen Stringfellow)

(but not entirely of them) are considered to fall into the same group. This category also includes the neoplasms such as squamous carcinoma previously considered to constitute "malignant transformation of mature cystic teratoma."

The following types of ovarian neoplasm are included: thyroid, carcinoid, central nervous system tumor, carcinoma, melanoma, sarcoma, sebaceous tumor, pituitary-type, retinal anlage tumor and others [17].

Thyroid Tumor Group

This is the commonest tumor in this group in the ovary. It is defined as a tumor that is composed predominantly (more than 50 %) or entirely of thyroid tissue [17], though it is recognized that some mature cystic teratomas containing a smaller proportion of thyroid tissue might need to be considered with this group because they have biologically active thyroid tissue or may include malignant thyroid elements, just as tumors composed of a higher proportion of thyroid may [17].

It is important to always bear this diagnosis in mind when confronted by a tumor with a predominantly tubular pathology – differential diagnosis includes Sertoli cell tumor or, in someone of an appropriate age, clear cell carcinoma of Mullerian type or even metastatic carcinoma of various sites. The presence of eosinophilic colloid should be of assistance (Fig. 14.11) (in at least some but not necessarily all of the tumors) as does positive immunocytochemistry for thyroid markers such as thyroglobulin.

Malignancy in Ovarian Thyroid Tissue

Forms with overtly malignant histology do exist. Though anaplastic strumal carcinomas do seem to follow a malignant course in the way that might be predicted from their histology [37], it is interesting and, perhaps, unexpected that interpretation of the histological features of many stromal tumors is not straightforward; for example, features that indicate papillary carcinoma in the thyroid do not necessarily correlate with a capacity to metastasize when identified in strumal tumors, and tumors with features that would be considered histologically benign in the thyroid gland may behave aggressively when seen in the context of struma ovarii [17, 37]. Therefore, it may be prudent to assume that behavior of these tumors cannot be predicted from histology.

Certainly, histologically benign tumors can show extra-ovarian spread. Peritoneal strumosis is the term given to benign-appearing peritoneal implants, and these do typically have an indolent course [38]. This diagnosis cannot be made if there is evidence of extraperitoneal spread or

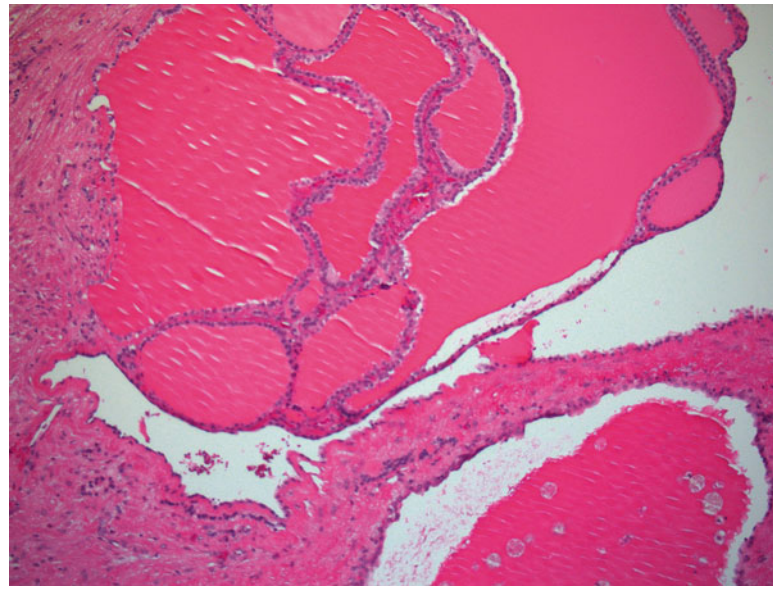

Fig. 14.11 Struma ovarii H&E ×100

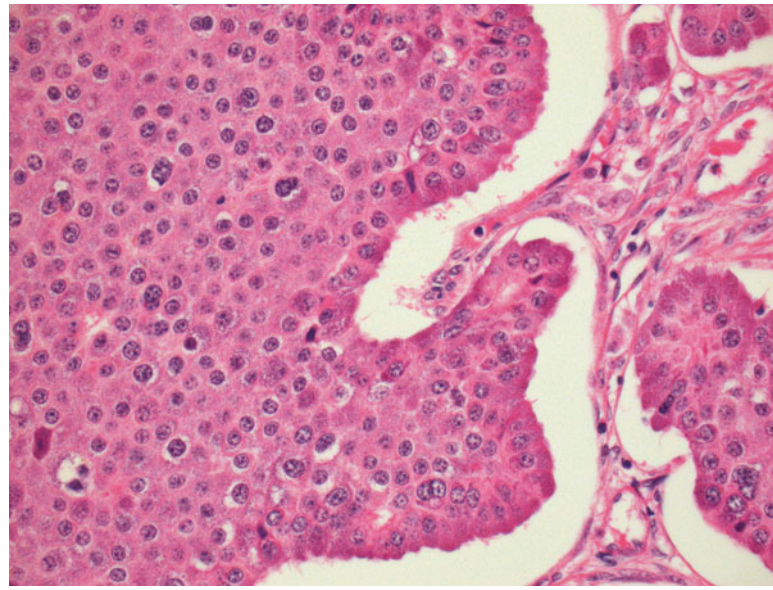

Fig. 14.12 Ovarian carcinoid with an insular pattern. H&E ×200

when follicular carcinoma is suspected or diagnosed in an ovarian strumal tumor [17], though as noted in the above paragraph, this is not an easy distinction to make on histological features alone and may require evidence of clinical progression [17].

Ovarian Carcinoid Group

Carcinoids are the next most frequent tumor in this group. Around 50–60 % of them have other teratomatous elements as part of the same tumor [17] and most have a midgut type, insular pattern (Fig. 14.12). Other patterns include trabecular tumors (Fig. 14.13) and mucinous carcinoids. The latter are less common and resemble goblet cell carcinoid of the type that arises in the appendix [17]. An obvious differential diagnosis for these three types when there are no other features of a teratoma is a metastatic carcinoid from a primary elsewhere. However, if bilateral, if there is a

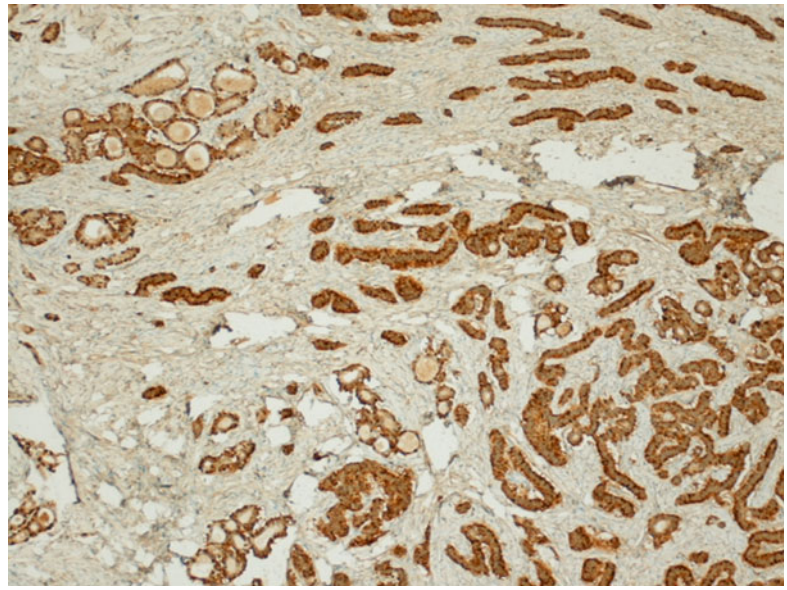

Fig. 14.13 Ovarian carcinoid with a partly trabecular pattern. Chromogranin ×100 (Photo provided by Dr Helen Stringfellow)

relevant history, and if there are multiple extra-ovarian metastases, then a metastatic lesion from a primary elsewhere is more probable than an ovarian primary [17].

Rarely the neoplasm known as strumal carcinoid occurs in the ovary – this is a tumor which consists of thyroid follicles mixed with groups of carcinoid cells; in one series of 50 cases, one patient died of the tumor, but in all other cases oophorectomy or salpingo-oophorectomy appeared to be effective treatment [39]. Extra-ovarian spread is very unusual in strumal carcinoids [17].

The carcinoid syndrome may be a feature of ovarian insular carcinoids [17]. Trabecular carcinoid can be associated with constipation due to peptide YY production [40].

Central Nervous System Tumor Type

Rarely there may be overgrowth of immature neural elements resembling neuroblastoma identified within an ovarian teratoma [41]. This pattern is associated with an aggressive course and poor prognosis [13]. Initially the term neuroectodermal tumor was applied to these neoplasms.

However, other forms of malignant neural tissue have been reported in ovarian teratomas, some of which appear to be primitive-type tumors and show features of medulloepithelioma, ependymoblastoma, or medulloblastoma [42], whereas others have features in keeping with glial differentiation and show features of ependymoma or glioblastoma [42]; the clinical course appears to be related to that of the tissue type, with glioblastomas having a malignant clinical course [13]. In one series, the age range was wide (6–69 years), though the average was in the early 20s, as for other tumors of probable germ cell origin [42]. Ependymoma has not been seen in association with other tumor teratomatous elements, leading to some doubts as to the validity of germ cell origin of this tumor in the ovary [17].

Carcinoma Group

Carcinoma is reported to develop in 1–3 % of teratomas, with squamous cell carcinoma the most common. The malignancy appears to develop *after* development of the teratoma that initially developed from a benign precursor cell [13]. Thus, in malignant transformation of ovarian MCT, there is a malignant clone developing within the background of a benign tumor. The malignant elements are homozygous, just as the original teratoma from which they developed [13].

Squamous Cell Carcinoma

Squamous carcinoma accounts for 80 % of the cases of carcinoma arising within MCT [43].

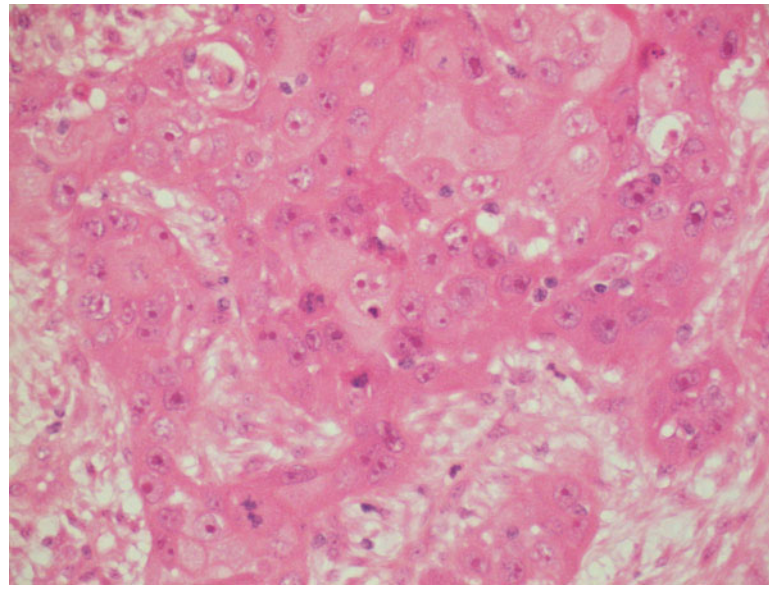

Fig. 14.14 Ovarian squamous carcinoma developing in association with a mature cystic teratoma. H&E ×200

Clinical Features As might be expected, malignant transformation is hardly ever reported preoperatively and is usually only diagnosed following histological assessment of the tumor. Women with malignant transformation may be slightly older than those with uncomplicated MCT; one small series noted a mean of 43 years for those tumors with malignant transformation, as opposed to a mean age of 32.6 years for the usual benign type of MCT [18]. An analysis of multiple papers on the subject suggested that the mean age may be over 50 years of age [43]. However, such tumors *may* occur occasionally in relatively young women, under 30 years of age.

Macroscopic Pathology The overall size of the tumor is extremely variable. The malignant element may be undetectable macroscopically or may form a large mass, breeching the ovarian surface. One series described the most common appearance of such tumors as cystic, ranging between 5 and 15 cm in maximum dimension [44]. Usually, therefore, the naked eye appearance is similar to an uncomplicated MCT. It goes without saying that it is prudent to histologically sample any area within the tumor that is of unusual or suspicious appearance.

Microscopic Pathology The histological features are those of a squamous carcinoma elsewhere (Fig. 14.14), but the size and degree of differentiation of the malignant element varies enormously from case to case. There are examples of tiny microscopic foci of invasive carcinoma adjacent to an area of in situ carcinoma [44]. Some cases may consist of large invasive tumors that penetrate the ovarian surface [44].

Prognosis Most series have identified that prognosis is dependent on stage, with tumors confined to the ovary and completely resected having a good prognosis [43–45]. Tumors that have spread outside the ovary have a poor prognosis, with very few survivors at 5 years follow-up [43].

Rarer Forms of Carcinoma A large range of tumor types have been described including mucinous adenocarcinomas [46] and pulmonary-type small cell carcinomas [47].

Sarcoma Group

Sarcomas account for approximately 8 % of malignancies occurring in association with a teratoma. Sarcomas of many cell types have been described [13]: angiosarcoma [48], rhabdomyosarcoma [49,

50], and osteosarcoma [51]. Carcinosarcoma has also been described [52].

Experience of such cases is exceedingly limited and therefore clinical significance is unknown.

Melanocytic Group

Malignant melanomas occasionally occur in the ovary [53]. While most such tumors are secondary, in some cases no other primary site can be identified and there is no history of melanoma excision. The tumors are therefore most probably ovarian primaries. One series described nine examples [53]. In six of them other elements of a teratoma were present either in the same or the contralateral ovary. Though some tumors contained no detectable elements of a teratoma, this does not exclude a teratomatous origin in which the other elements were effaced; in such circumstances it can be difficult both to make the diagnosis, given the notorious capacity of melanoma to mimic other tumors, and also to decide that the neoplasm is truly an ovarian primary [53]. Clinicopathological correlation is the best way to try to approach this – but it may prove impossible to judge with certainty.

Benign and atypical melanocytic lesions have also been described in mature cystic teratoma [54].

Sebaceous Tumor Group

Sebaceous tumors are reported in the ovary (some with a component of basal cell carcinoma) [55]. The prognosis seems favorable in most, but not all, cases [17].

Pituitary-Type Tumor Group

Pituitary-type tumors have been reported in association with other elements of a mature cystic teratoma. These tumors may secrete hormones and therefore may be associated with relevant clinical effects. Both prolactinoma [56] and corticotroph cell pituitary-type adenoma [57] have been reported.

Retinal Anlage Tumor Group

Retinal anlage tumor in association with ovarian teratoma is described and can behave aggressively [58], though benign examples are also reported [17]. The histological features are similar to retinal anlage tumors elsewhere, with two cell types – larger melanin containing cells and smaller undifferentiated cells that have no pigment and resemble neuroblastoma.

Other Forms of Tumor Postulated to Be of Teratomatous Origin

Neoplasms that are entirely or almost entirely vascular are reported, often occurring in children and young adults [59]. The constituent cells are described as showing cellular and nuclear pleomorphism with mitoses [59] or sometimes bearing some similarity to hemangiopericytoma [59]. The differential diagnosis lies with angiosarcoma [48] or with the florid vascular proliferation occasionally associated with the neural component of teratoma (both mature cystic teratomas and immature teratoma [60]). Ovarian vascular tumors have been described with a mature cystic teratoma in the contralateral ovary [61].

Other very unusual tumors have occasionally been reported – for example, chordoma has been described in the ovary [62]. Nephroblastoma-like overgrowth of primitive renal tissue has been reported [63]. Lymphoma has been described [64].

Primitive Germ Cell Tumors

While ovarian teratomas are a distinct group in which a counterpart neoplasm in the testis is very unusual, primitive germ cell tumors of the ovary share many features in common with their testicular counterparts. Most show the characteristic cytogenetic abnormality of 12p amplification (not seen in ovarian teratomas, whether mature or immature) [13]. The histological features are identical in the two locations, though there are marked differences in incidence between the various histological patterns.

Seminoma/Dysgerminoma/Germinoma

A tumor with similar biological features is known as seminoma in the testis (where it is the most common germ cell tumor), dysgerminoma in the ovary or in dysgenetic gonads, and germinoma in the brain. The cells have similar features to PGC. It is the second most common germ cell tumor of the ovary (though accounts for only 2–3 % of them).

Clinical Features

It predominantly affects younger women with 85 % of patients being less than 30 years at diagnosis [65], though cases are reported from 7 months to 70 years [59] so that it can occur both before puberty and after the menopause, but the great majority occurs in later childhood, adolescence, and in young adults. Dysgerminoma has been reported in siblings and in a mother and daughter [59].

Presentation is usually with abdominal pain or distension [65]. Like other ovarian masses it may present acutely with torsion, when the histological diagnosis may be difficult because of the extent of necrosis and hemorrhage. The presence of high human chorionic gonadotrophin (hCG) (see below) can lead to hormonal manifestations including abnormal bleeding (precocious puberty in children) or hyperthyroidism (because hCG has a thyroid-stimulating-like activity [13]). There are also reports of dysgerminoma presenting with paraneoplastic limbic encephalitis [66] and with the manifestation of paraneoplastic hypercalcemia [67]. It may be discovered during investigations for primary amenorrhea because of the association with disorders of sex development and dysgenetic gonads, discussed later.

Macroscopic Features

Dysgerminoma is more frequently bilateral than other malignant germ cell neoplasms – one recent series identified 6.5 % bilateral tumors [68], though others have suggested it may be nearer 15 % [59]. Dysgerminomas are usually solid tumors, cream to tan in color, ranging in size from a few cm to very large. There may be focal hemorrhage (Fig. 14.15). Usually there is an intact capsule, but this can rupture in some cases. Small cysts may be present, though this is unusual in pure dysgerminoma [59]. If there is a cystic area, it may be prudent to sample this thoroughly when selecting tissue for histological analysis, since the presence of other malignant germ cell elements can be of prognostic importance.

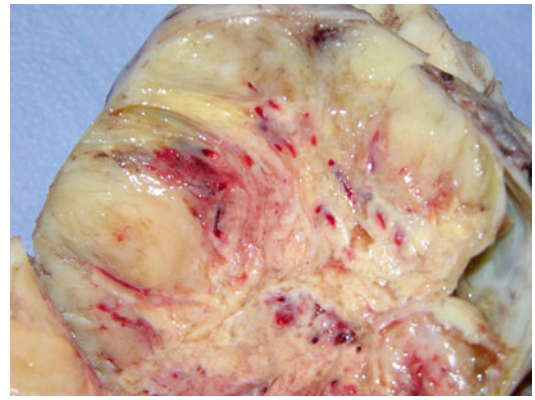

Fig. 14.15 Ovarian dysgerminoma, macroscopic appearance (Photo supplied by Dr Helen Stringfellow)

Microscopic Features

Typical examples are very easy to identify with sheets of large cells with clear cytoplasm and well-defined cytoplasmic borders separated into aggregates by fibrous septa (Fig. 14.16). They have a large vesicular nucleus with a prominent nucleolus. Usually the fibrous septa are fine but may be much more dense. Typically there is an associated accumulation of T lymphocytes and histiocytes [17] (Fig. 14.17), but this may be absent (Fig. 14.18). In 25 % of cases epithelioid granulomas are also seen within the accompanying inflammatory cells [17]. Dysgerminomas show mitotic activity, but the rate varies considerably, between and within tumors. Syncytiotrophoblast cells are present in a minority of tumors and this results in hCG production, as described above. Unless there is cytotrophoblast seen in association with the syncytiotrophoblast, this does not imply that the tumor is actually a mixed germ cell neoplasm with a component of choriocarcinoma. The syncytiotrophoblast cells will, in whatever context, produce and stain immunochemically for hCG. Dysgerminoma cells contain cytoplasmic glycogen so are PAS positive. Calcification is unusual. Sometimes there may be a calcified structure in the background, suggestive of a previous gonadoblastoma, and this should prompt the clinical and laboratory search for a disorder of sex development (DSD), if this was not previously evident.

Differential Diagnosis

Some of the characteristic features may be absent in certain examples, leading to confusion with

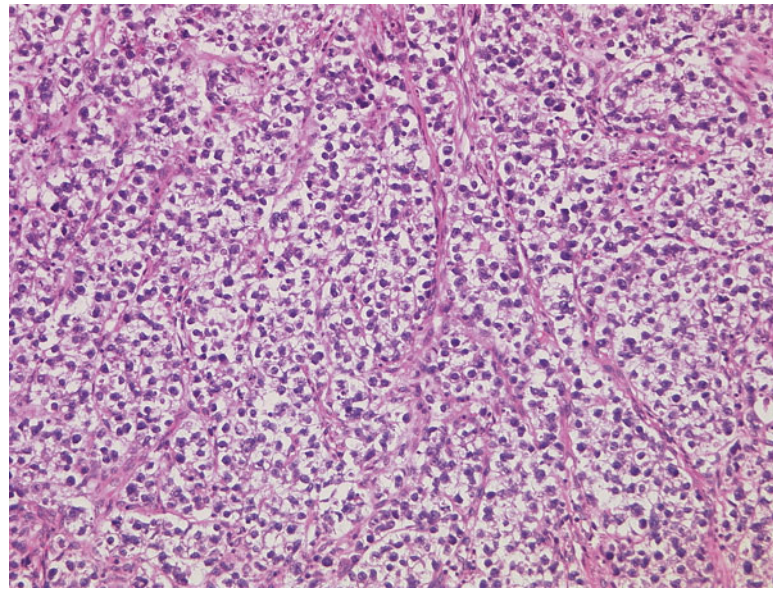

Fig. 14.16 Dysgerminoma. Cells separated by fine collagen bands. H&E ×100 (Photo provided by Dr Nafisa Wilkinson)

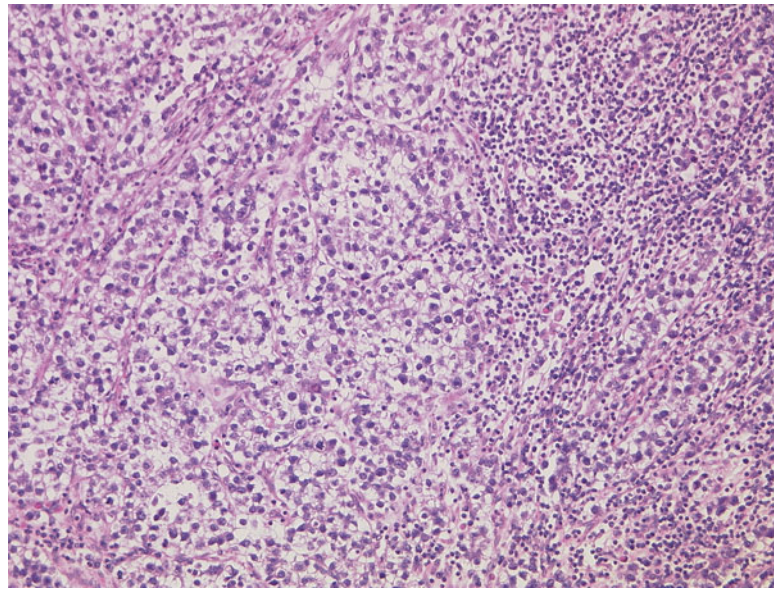

Fig. 14.17 Dysgerminoma with typical lymphocytic aggregates H&E ×100 (Photo provided by Dr Nafisa Wilkinson)

other germ cell tumors, such as yolk-sac tumor or embryonal carcinoma. Furthermore, the cytological features of the dysgerminoma cells or their arrangement may show atypical features. Ulbright documents this in detail in his extremely helpful review covering germ cell tumors as a whole [13]. Dysgerminomas may rarely have a microcystic or cribriform pattern, leading to confusion with yolk-sac tumor. However, immunocytochemistry should be helpful in sorting out this particular dilemma (see specific section below).

Somatic Differentiation in Dysgerminoma

Rarely a tumor with the features of a typical dysgerminoma may also show features of somatic differentiation. Tumors with a rhabdomyosarcomatous component [69] and also with a fibrosarcoma [70] have been reported. The latter was

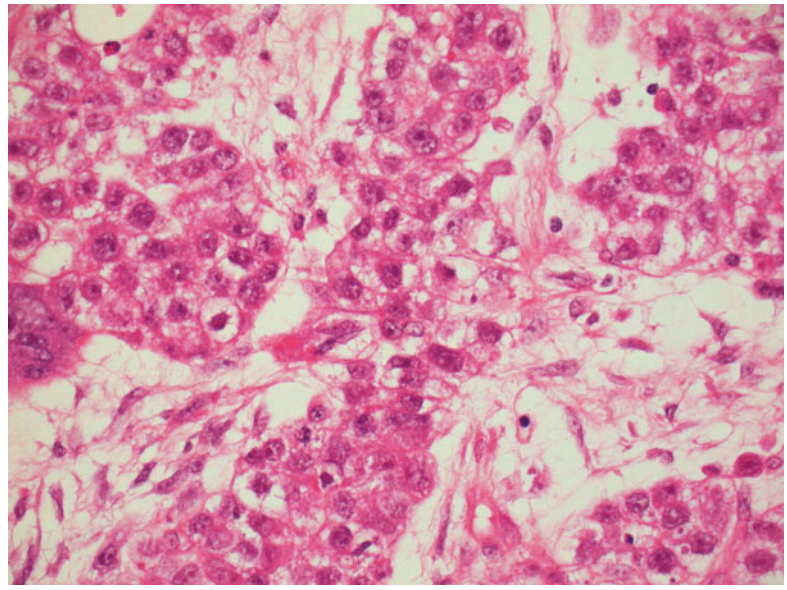

Fig. 14.18 Dysgerminoma – no lymphocytes – differential diagnosis is with other more malignant germ cell tumor types, yolk-sac tumor and embryonal carcinoma H&E ×200

associated with a poor response to treatment and rapid fatality [70].

Prognosis

Approximately 75 % of women with dysgerminoma present with clinical stage I disease [65], though the duration of symptoms is usually short, indicating probable rapid growth. Tumors are very radiosensitive and are sensitive to chemotherapy so that the prognosis is excellent, even with the unusual tumor that is disseminated at diagnosis [65]. Overall 5-year survival is over 90 %. Even in the pre-chemotherapy days, prognosis for early stage cancers was very good with conservative surgery alone [65].

Yolk-Sac Tumor

This is the third most common form of ovarian germ cell tumor, where it is usually a pure tumor, in contrast to other sites where (in the postpubertal individual) it is usually part of a mixed germ cell tumor.

Yolk-Sac Tumor in General and Histology

Yolk-sac tumors are a multifaceted group of neoplasms that differ radically in biology in the various sites where they occur [13]. However, all yolk-sac tumors have areas with the morphology of primitive extra embryological tissues analogous to the early stages of embryonic and extra-embryonic development [15]. The constituent cells are large, are pleomorphic, and may have vesicular or hyperchromatic nuclei. The cytoplasm varies – it may be clear or eosinophilic and may contain the characteristic PAS-positive, diastase-resistant hyaline globules. Pale eosinophilic extracellular basement membrane material is a characteristic feature of yolk-sac tumor. The appearance of the cells may vary in different parts of the tumor. They may be flattened. When lining cysts, the tumor cells may protrude into the cyst, giving a "hob-nail" appearance. Histological patterns resembling the extraembryonic elements include reticular-microcystic, endodermal sinus (with the characteristic Schiller-Duval sinus) (Fig. 14.19), parietal (resembling mouse parietal yolk sac and AFP negative [15]), polyvesicular, and tubular [15]. The appearance therefore may be predominantly glandular in appearance (Fig. 14.20). Hematopoiesis can be present in these tumors [15]. Yolk-sac tumors may also display a number of histological patterns analogous to endodermal somatic tissues such as respiratory, intestinal, and hepatic tissue closely resembling fetal liver [15]. Solid forms are described [15] as are tumors with mesenchymal overgrowth

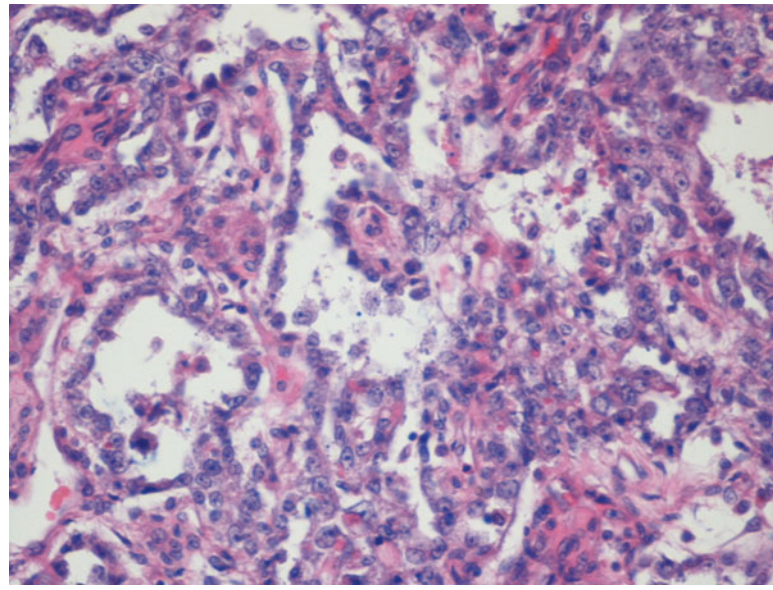

Fig. 14.19 Yolk-sac tumor. Schiller-Duval sinus. H&E ×200 (Photo supplied by Dr Helen Stringfellow)

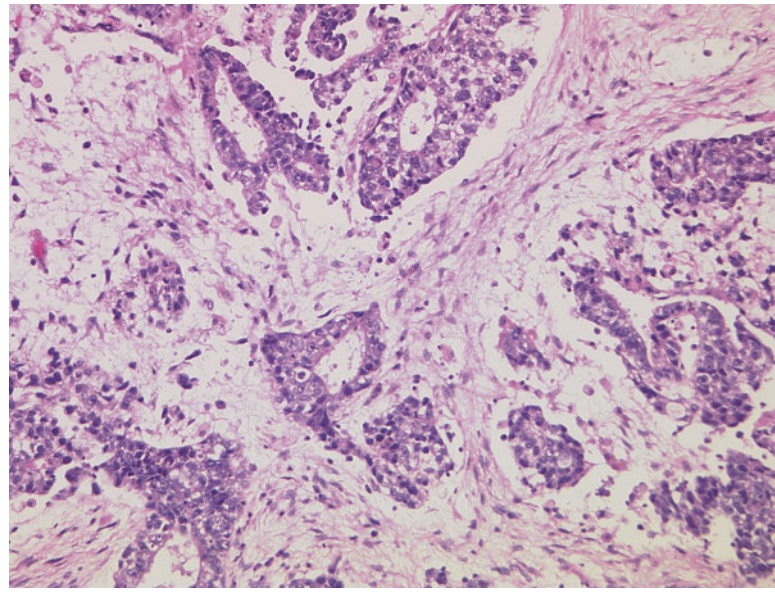

Fig. 14.20 Yolk-sac tumor. Glandular area resembling carcinoma. H&E ×100 (Photo provided by Dr Nafisa Wilkinson)

and few islands of primitive epithelium (Fig. 14.21) [15]. The mesenchymal component can be so extensive as to make diagnosis difficult, because of the paucity of epithelial elements. Hemorrhage is common (Fig. 14.22).

The Schiller-Duval body is present in a minority of tumors only [71]. In most tumors typical areas are present. The periodic-acid-Schiff (PAS)-positive hyaline globules, found in AFP synthesizing cells, are an important aid to diagnosis in an appropriate clinical and pathological setting and if there is evidence of AFP production (Fig. 14.23) [71].

At times, this mesenchymal component can undergo sarcomatous change, particularly following obliteration of the epithelial elements by chemotherapy [15].

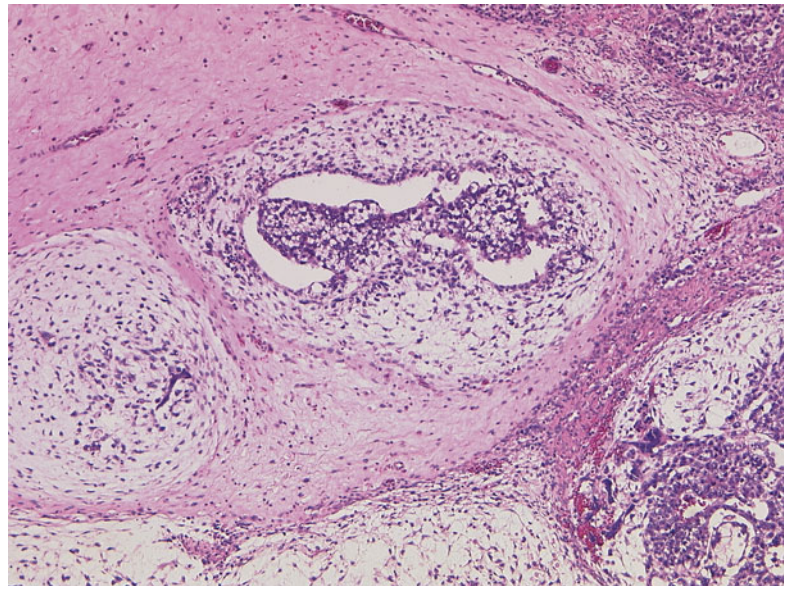

Fig. 14.21 Yolk-sac tumor – area with abundant mesenchyme and only a few islands of primitive epithelium. H&E ×2,100

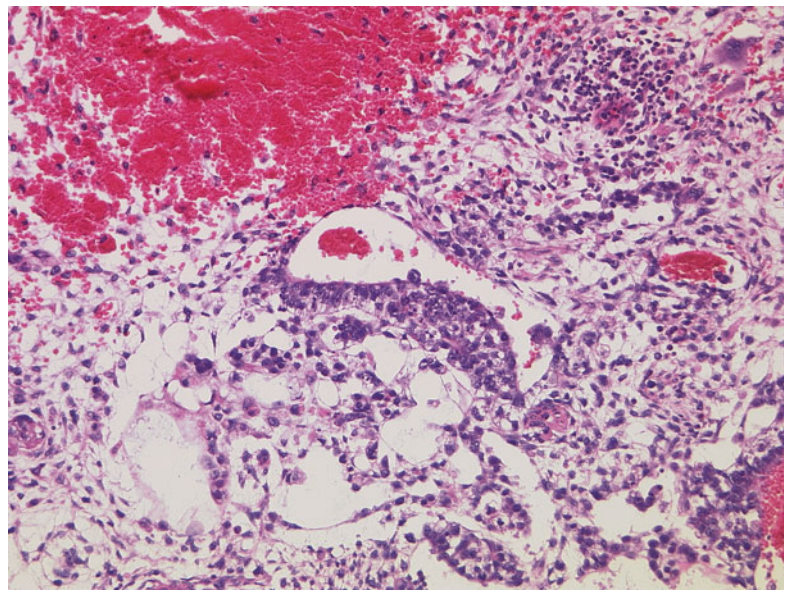

Fig. 14.22 Yolk-sac tumor – hemorrhagic area H&E ×200

Clinical Features

Yolk-sac tumors differ radically in biology at various sites, but ovarian tumors were highly malignant and almost universally fatal before the advent of modern chemotherapy. For example, in a large series from the armed force institute in the 1970s [72], survival was 13 % at 3 years. The introduction of cisplatin into the oncological armory has transformed yolk-sac tumor (YST) from a fatal to a curable tumor [73].

It is a rare tumor, comprising only about 1 % of all ovarian malignancies [73]. As for other malignant germ cell neoplasms, the most frequent age group affected are young adults, with occasionally cases involving children and young teens. One study found a median age of 18–25 years, with one patient aged as young as 5 years at diagnosis [71]. Occasional patients are postmenopausal [74], though this group may be clinically distinct – discussed below. Clinical

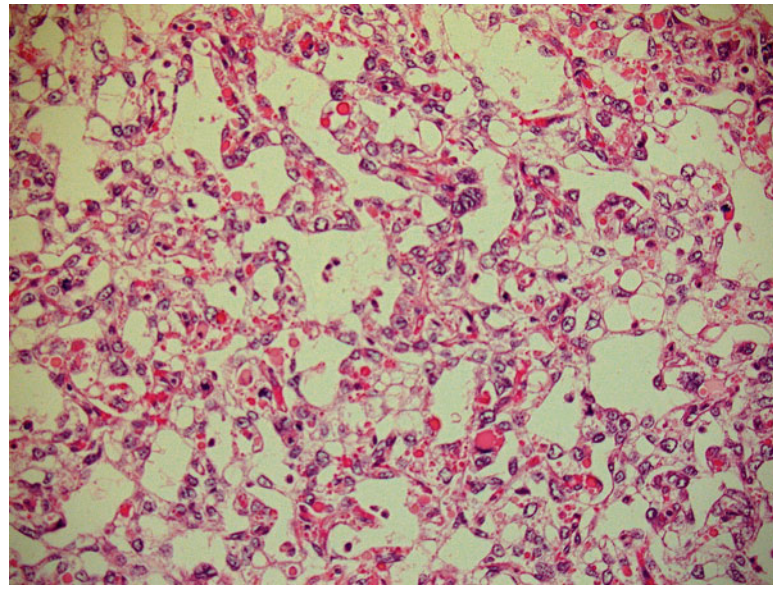

Fig. 14.23 Yolk-sac tumor. Hyaline globules. Here photographed in H&E stain. ×200

presentation is most commonly with abdominal pain, abdominal enlargement, and abdominal or pelvic mass. Fever is present in a quarter of cases; some present with acute abdominal symptoms as a result of torsion or rupture [71]. At presentation, 60–70 % of yolk-sac tumors are confined to the ovary, with stage IV (FIGO) being very uncommon [71]. It is rare but not impossible for yolk-sac tumor to present with hormonal manifestations; the stroma adjacent to the tumor may produce endocrine effects [71].

Alpha-fetoprotein is usually raised in serum, assisting diagnosis (though as discussed below, other non-germ cell tumors can also show this), and may also assist follow-up following surgery and other treatment [71].

Macroscopic Appearance

It is most frequently a unilateral tumor of young women [73]. Bilateral ovarian involvement is very uncommon [71] but not unknown [74], though it may be a manifestation of metastasis, rather than indicative of bilateral primary tumors. Most usually yolk-sac tumors are large and rapidly enlarging, with a median diameter of 15–19 cm [71]. They may be encapsulated, but herniation or rupture may occur. The cut surface may be cystic, but usually there are at least some solid areas, ranging in color from white to yellow to brown. Usually there is necrosis and hemorrhage.

Differential Diagnosis

There are several possible differential diagnoses to consider, and Ulbright describes these in detail in his review of germ cell histopathology [13].

One of the most important and difficult distinctions is between clear cell carcinoma (Fig. 14.24) and yolk-sac tumor of the ovary. Histologically, there may be clues; yolk-sac tumors commonly have intracytoplasmic hyaline globules and extracellular basement membrane deposits. The presence of other germ cell elements may provide useful evidence in the mixed germ cell tumor. Obviously, there may also be differences in the clinical and other laboratory features such as the age of the patient (often – not always – significantly older in clear cell carcinoma) and level of serum alpha-fetoprotein (AFP) in most cases; however, raised serum AFP has been reported in ovarian clear cell carcinoma [75] Some yolk-sac tumors can be predominantly glandular with subnuclear vacuolation, and therefore they resemble endometrioid carcinoma. Immunocytochemistry should be helpful and this is discussed below.

There is a further complication in that a clinically distinct form of yolk-sac tumor can rarely

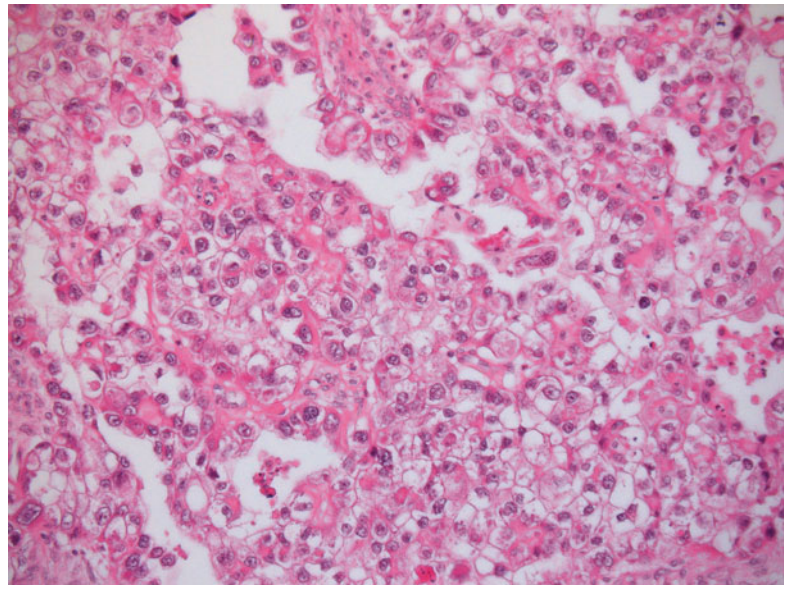

Fig. 14.24 Ovarian clear cell carcinoma H&E ×200

occur in older women [13, 76]. Just as in conventional yolk-sac tumor of germ cell origin, the tumors produce AFP and may show immunocytochemical positivity. In some cases there is an epithelial component, such as a clear cell or endometrioid carcinoma present, but this is not always so [76]. From the cases so far studied, it appears that this is a tumor that is clinically quite distinct from the typical yolk-sac tumor of young women. There is less response to conventional germ cell therapy, and it has been suggested that therapy designed to treat both germ cell neoplasms and epithelial tumors may be more appropriate in this context [76].

In yolk-sac tumors with hepatoid areas, there is a another differential diagnosis to be considered. Differentiation along hepatic lines is not commonly seen in other ovarian tumors, with the exception of the rare hepatoid carcinoma – which usually occurs in an older age group and which is usually accompanied by conventional adenocarcinoma [13, 77].

Yolk-sac tumor may sometimes mimic other germ cell tumors [13], and here, a panel of immunostains might help (as tabulated below), and, in at least some areas, there is likely to be typical yolk-sac pattern, hence the need for careful histological sampling in neoplasms in this group.

Prognosis and Treatment

Treatment is initially surgical, but with fertility preservation wherever possible. Modern chemotherapy has led to a vast improvement in survival so that survival for tumors diagnosed at an early stage is now good (95 % 5-year survival in one study [71]).

Embryonal Carcinoma

Though relatively common in the testis (10 % of germ cell testicular tumors), embryonal carcinoma is rare in the ovary in its pure form [13]. It is much more frequently seen as part of a mixed malignant germ cell neoplasm, often in association with yolk-sac tumor. Therefore, the age range, presentation, and macroscopic appearance are exactly the same as those for other germ cell tumors. AFP may be elevated, even if there are no yolk-sac elements in the tumor, but the elevation is usually only moderate [59]. It can contain syncytiotrophoblast or can be combined with choriocarcinoma so can present with the hormonal manifestations (abnormal bleeding or precocious puberty) associated with raised hCG [59].

Microscopic Features

The constituent cells are large and pleomorphic with prominent mitoses. There may be small solid islands of cells or there may be pseudoglandular

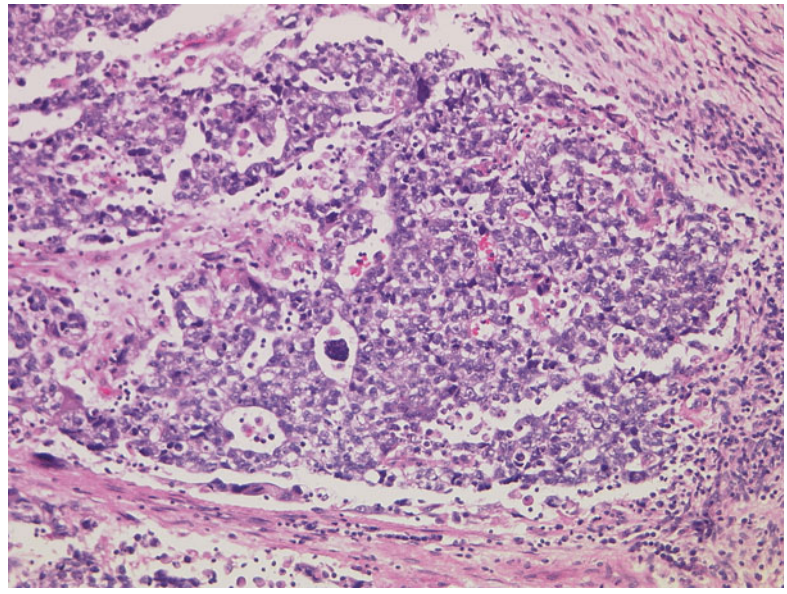

Fig. 14.25 Ovarian embryonal carcinoma H&E ×200

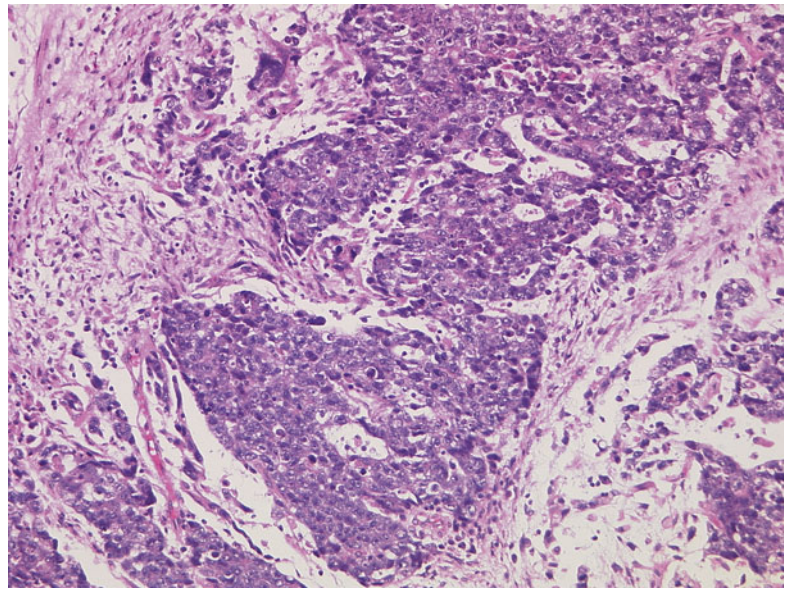

Fig. 14.26 Ovarian embryonal carcinoma H&E ×200

areas (Figs. 14.25, 14.26, and 14.27). The most difficult differential diagnosis in the context of a malignant germ cell neoplasm is dysgerminoma as there are similarities in the H&E appearance of the constituent cells (compare with Fig. 14.17). The lymphoid infiltrate, typical of dysgerminoma, is not usually seen in embryonal carcinoma. Furthermore, there are immunocytochemical differences, explained below. It is important to distinguish between dysgerminoma and embryonal carcinoma, since the latter is highly malignant and associated with early metastases [59].

The diagnosis can be difficult when embryonal carcinoma presents as a metastatic lesion, rather than as a primary. This diagnosis needs always to be considered when assessing a

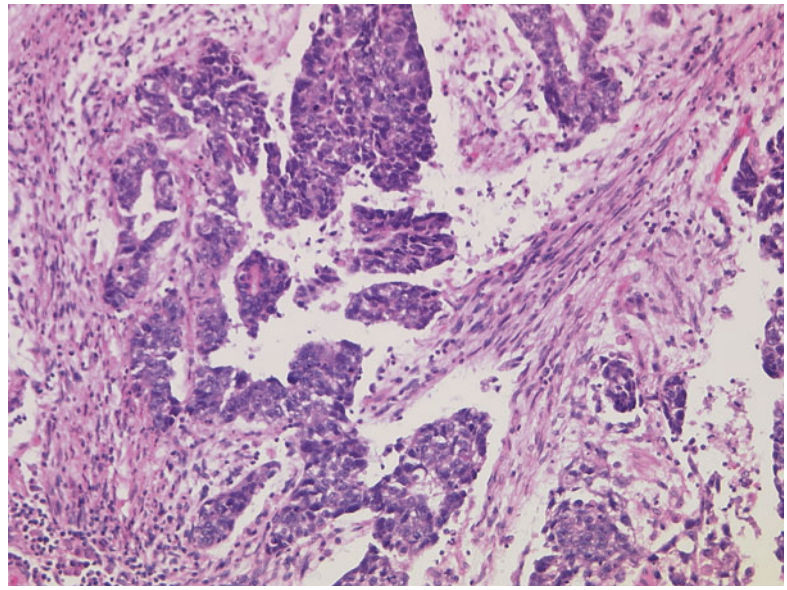

Fig. 14.27 Ovarian embryonal carcinoma H&E ×200

metastasis in any organ from an unknown primary site, particularly in a younger woman, where the differential will include lymphoma, melanoma, and other forms of carcinoma, in addition to very rare entities. Its immunoprofile is distinct *in this context* (see section below).

Choriocarcinoma

This is one of the least common of gonadal germ cell tumors, certainly in its pure form, unaccompanied by other malignant germ cell elements.

In the ovary, the obvious diagnostic dilemma is between primary and metastatic gestational choriocarcinoma. The latter will usually be associated with a recent or current gestation [13]. For non-gestational choriocarcinoma, the clinical features are the same as those for other malignant germ cell tumors. Hormonal effects also are frequent. It produces hCG and therefore may be associated with hormonal manifestations such as precocious puberty or abnormal bleeding in postpubertal individuals or hyperthyroidism because of the additional effects of hCG. These are similar to those sometimes associated with dysgerminoma or embryonal carcinoma, for the same reason.

Macroscopically, choriocarcinoma is likely to be hemorrhagic with areas of necrosis [59]. Microscopically, it is composed of two cell types, the mononuclear and the multinucleate trophoblast. The mononuclear cells are medium-sized, polygonal cells with clear cytoplasm and sharp borders. The nuclei can be small round and hyperchromatic or large and vesicular [59]. The multinucleated cells are syncytiotrophoblast – basophilic, vacuolated cells with multiple hyperchromatic nuclei [59] (Fig. 14.28). Hemorrhage is a particularly common finding in choriocarcinoma (Fig. 14.29), so that this finding in a malignant germ cell tumor in which the predominant tumor type is one of the other types or mixed means that a careful search for choriocarcinoma is warranted, though other types may also be associated with hemorrhage (e.g., see Fig. 14.22).

Clinically, non-gestational choriocarcinoma is malignant, but modern chemotherapy with cisplatin has revolutionized the prognosis [59].

Mixed Germ Cell Tumors

Though these represent 50 % of germ cell neoplasms in the testis, they form only 1 % of ovarian germ cell tumors [13]. As might be expected they are formed by a mixture of the elements described above. These tumors may include elements of a teratoma, and it seems, on the basis of a high incidence of abnormalities of chromosome 12p, that in this situation the teratoma forms from a malignant precursor cell, analogous to the postpubertal testicular teratoma [78]. They are

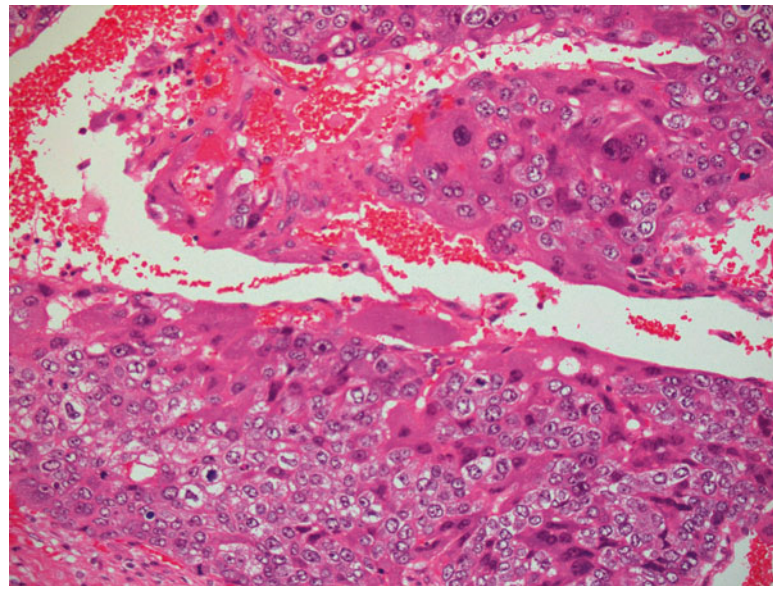

Fig. 14.28 Choriocarcinoma H&E ×400

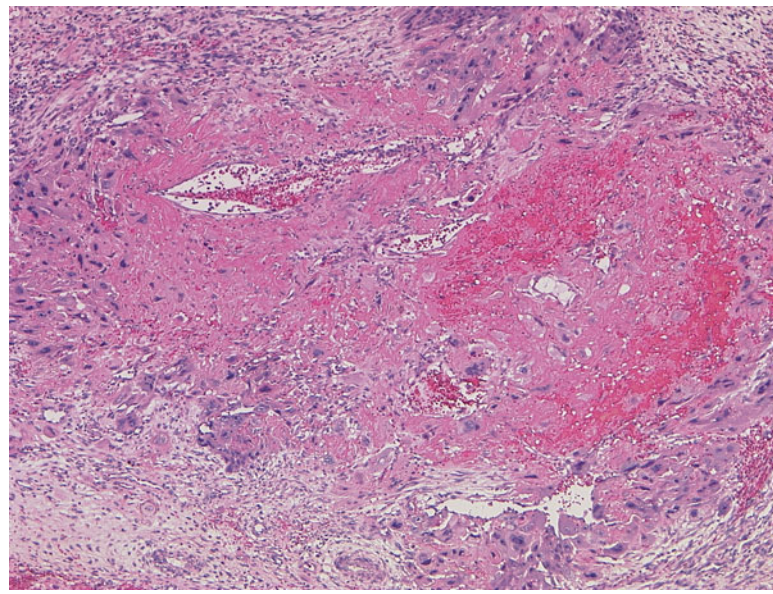

Fig. 14.29 Choriocarcinoma H&E ×200

different in pathogenesis to the other forms of ovarian teratoma.

Immunocytochemistry in Primitive Germ Cell Tumors (See Table 14.1)

Germ Cell or Other Tumor?

SALL4 is a stem cell marker in the same family as OCT3/4 that seems to regulate OCT3/4 transcription. It is a zinc finger transcription factor that shares homology with the Drosophila *spalt* (*sal*) gene. In Drosophila, this gene plays an important role in specifying the head and tail [79]. It is a useful marker indicative of germ cell differentiation particularly when the differential diagnosis includes epithelial or sex cord-stromal tumors [80]. The nuclei of normal oocytes are positive for SALL4 [80]. It is expressed in the nuclei of a wide range of tumors of germ cell

Table 14.1 Some of the more helpful immunocytochemical markers to help distinguish between types of malignant germ cell tumors

	Ae1/Ae3 (memb)	AFP (Cyt)	Oct3/4 (nuclear)	CD117 (memb, cyt)	PLAP (memb, cyt)	CD30 (memb)	EMA	SALL4 (nuclear)	D2-40 (mem, cyt)	Glypican-3 (cyt)	SOX-2 (nuclear)
Dysgerminoma	May be pos	NEG	+	+	+	NEG	NEG	+	+	NEG	NEG
Yolk-sac tumour	+	+	NEG	May be pos	+	NEG	NEG	+	NEG	+	NEG
Embryonal carcinomas	+	+/-	+	NEG	+	+	+	+	NEG	NEG	+

Markers that are particularly useful in this context are shaded in red. OCT3/4, SALL4, and SOX-2 are nuclear markers, whereas keratins, AFP, CD117, PLAP, CD30, EMA, D2-40, and glypican-3 are cytoplasmic/membrane markers. SALL4 is particularly helpful in distinguishing between a tumor of germ cell origin where the differential is between germ cell and non-germ cell tumors

origin – including dysgerminoma, germ cells in gonadoblastoma, embryonal carcinoma, and yolk-sac tumor, though probably not choriocarcinoma [80]. However, it can be expressed weakly in a minority of clear cell carcinomas and also some carcinomas from other sites [80] so care is needed in interpretation.

PLAP can also be used to identify germ cell neoplasms, since it is expressed in dysgerminoma (and gonadoblastoma), embryonal carcinoma, yolk-sac tumor, and choriocarcinoma [80]. However, expression is variable, and some ovarian epithelial tumors can express it [81] so there are limitations to its usefulness. It is also not a nuclear marker and staining may be harder to interpret than the very clear nuclear positivity of SALL4.

If the differential diagnosis is between a germ cell tumor and a sex cord-stromal tumor, then the latter express calretinin, inhibin, SF-1 (a transcription factor that regulates differentiation), and FOXL2 (a transcription factor that governs granulosa cell function), whereas germ cell tumors do not do so and sex cord-stromal tumors do not express PLAP or SALL4 [80, 82].

c-KIT is expressed in many other tumors and cell types and cannot be used to indicate germ cell lineage. Expressions of function of c-KIT and gain of function of *c-KIT* have been found in mastocytosis, leukemia, malignant melanomas, and gastrointestinal stromal tumors [6].

Immunocytochemistry of Individual Germ Cell Tumor Types

Dysgerminoma cells are reported to stain positively for vimentin, NSE, PLAP, CD117 (c-KIT), OCT3/4, NANOG protein (product of a stem cell gene), and D2-40 (also known as podoplanin) [83]. OCT3/4 is also expressed by the germ cells in gonadoblastoma and by embryonal carcinoma cells, and this marker is *very specific* for these tumor types and therefore extremely helpful, when used as one marker in a panel. CD117 is less useful in specifically identifying dysgerminoma among other germ cell tumors as it can be expressed in some solid yolk-sac tumors [84]. It may also be expressed by some serous carcinomas [85]. Keratins (Cam 5.2, AE1/AE3, and CK7) can be expressed by a minority of dysgerminomas, but EMA is not expressed [80]. D2-40 (podoplanin) can also be helpful if the differential is between dysgerminoma and other malignant germ cell tumors, since it is expressed in dysgerminoma but not in the other tumor types [86].

Yolk-sac tumor is the neoplasm, the diagnosis of which is most likely to prove problematic. However, immunocytochemistry can be helpful.

Because ovarian clear cell carcinoma is often the most important differential, it is important to note that SALL4 should be expressed in yolk-sac tumor as in most other germ cell tumors, but not in clear cell carcinoma (though there may be some weak staining). For this differential diagnosis, this is the immunostain most likely to prove helpful assistance.

Previously, AFP was considered a helpful marker. Yolk-sac tumors should be positive for AFP and also for alpha 1 antitrypsin, cytokeratin, CD 34, and CEA. However, the cytoplasmic staining of AFP can be weak and patchy, particularly in the solid variant of YST, and the overall sensitivity has been stated to be as low as 60 % [80]. The major limitation to the use of AFP alone to identify yolk-sac tumors is that up to a third of clear cell carcinomas can express AFP, further evidence that a panel of immunostains including those most specific to germ cell differentiation is required [79]. Glypican-3 has been demonstrated in the cytoplasm of many yolk-sac tumors [80]. However, glypican-3 is of uncertain practical use as a marker in this context, since it can be patchy in yolk-sac tumor and it can also show positive staining in ovarian clear cell carcinoma [79].

As far as distinguishing between yolk-sac elements and other forms of germ cell neoplasm, unlike dysgerminoma and embryonal carcinoma, yolk-sac tumor is negative for OCT3/4 [80]. Though in the context of other germ cell neoplasms the specificity of AFP for yolk-sac tumor is high, it *can* be expressed by other germ cell tumor types; embryonal carcinoma and enteric or hepatic elements in a teratoma may be positive [80]. Glypican-3 could also be helpful in a panel to distinguish yolk-sac elements from dysgerminoma or embryonal carcinoma, since yolk-sac tumor is the only tumor among these three to express it [80].

Embryonal carcinoma. Much of the published work deals with the analogous testicular neoplasm rather than the ovarian tumor, but the pathology is identical [80]. OCT3/4 is expressed. CD30 is a member of the tumor necrosis factor receptor superfamily that was initially identified as a surface marker for the malignant Reed-Sternberg cells of Hodgkin disease [87]. It is expressed in only a few cell types including activated lymphocytes and decidual cells, but the list includes the majority of human embryonal carcinoma cells, and it is therefore a useful marker indicating this rare line of differentiation, since other germ cell tumors should not express it [80].

SOX 2 is one of the regulatory factors associated with pluripotency [88]. SOX2 nuclear expression is present in embryonal carcinoma, but not in dysgerminoma, and so can also be useful when this is the differential diagnosis though some epithelial tumors express it [80].

Choriocarcinoma. Choriocarcinoma is usually easy to recognize from its microscopic appearance. However, immunocytochemistry can be used to help and confirm. The syncytiotrophoblastic cells express hCG (but not the cytotrophoblast), but the presence of the syncytiotrophoblast does not of course make the diagnosis of choriocarcinoma, since these cells may be present in other malignant germ cells. The latter do express cytokeratin, inhibin, and glypican-3 [81].

Carcinoid Tumors

Ovarian carcinoids may cause diagnostic problems with other ovarian primary tumors, for example, granulosa cell tumor. Carcinoids, as elsewhere, express synaptophysin and chromogranin [80]. CK7 and pancytokeratin can be positive, but EMA is usually negative, whereas adenocarcinomas will usually express EMA [80]. Work is underway to try to identify a means of distinguishing primary carcinoids from metastatic tumors. Positive TTF-1 suggests a metastasis from a primary pulmonary carcinoid, whereas PAX8 is expressed by pancreatic, gastric, duodenal, appendiceal, or rectal carcinoids and metastases, but initial studies suggest it is not expressed by primary ovarian carcinoids [80].

Estrogen Receptors

This is not an area that has been fully explored as yet, possibly because of the rarity of malignant ovarian germ cell tumors. Because of the peak age

group at which malignant ovarian germ cell tumors (MOGCTs) develop, often soon after puberty, usually at an age of less than 20 years, but in any case usually during child bearing years, it may seem probable that gonadal steroids may have some role in their development. While estrogen receptors (ERβ (beta)) and co-regulators are downregulated in testicular seminomas, embryonal carcinomas, and mixed germ cell tumors, in one series of MOGCT tested, all expressed estrogen receptors and their co-regulators – including small nuclear RING finger protein (SNURF/RNF4). However, as yet the significance of this finding is unknown and it has not yet been translated into clinical practice [89].

DSD and Dysgenetic Gonads

Normally gonads develop along either the testicular or the ovarian pathway, and this is strongly tied to the nature of the sex chromosomes, so that an ovary usually develops when there is a 46,XX karyotype and a testis normally develops when the chromosome complement is 46,XY. However, the process is very complex, and there are many possible deviations from the normal state. There are many conditions of incomplete or disordered genital or gonadal development leading to discordance between genetic sex (meaning the X and Y chromosomal constitution), gonadal sex (the testicular or ovarian development), and phenotypic sex (the physical appearance of the individual). Some individuals may possess both testicular and ovarian tissue, either in a single gonad or in two different gonads. Other situations may be characterized by the development of unilateral or bilateral streak gonads.

The group of conditions in which this occurs was previously known as hermaphroditism or intersex, but it has been referred to as *disorders of sex development* (DSD) since 2006 [90].

Classification of DSD

Previously, classification was on the basis of the gonadal tissue present, hence the use of terms such as "mixed gonadal dysgenesis" or "true hermaphrodite." Clearly this had the problem that it necessitated gonadal biopsy (or in the early days, autopsy). DSDs are now classified by the karyotype of the individual concerned, resulting in three basic groups [7]:

1. *Sex chromosome DSD*. This includes those with numerical sex chromosome anomalies, such as 47,XXY (Klinefelter syndrome and variants); 45,X (Turner syndrome and variants); and 45,X/46,XY mosaicism (formally mixed gonadal dysgenesis).
2. *46,XY DSD*. This includes all patients with:
   (a) *Disorders of testicular development*. This includes:
      (i) Complete and partial and partial gonadal dysgenesis (due to mutations of genes required for testicular development such as SRY, SOX9, SF1, WT1, and so on)
      (ii) Ovotesticular DSD
      (iii) Testicular regression
   (b) *Disorder of androgen synthesis or action*
3. *46, XX DSD*. This includes all patients with:
   (a) *Disorders of ovarian development*. This includes:
      (i) Gonadal dysgenesis
      (ii) Ovotesticular DSD
      (iii) Testicular DSD (due to the combination of factors present – SRY presence, duplication of SOX9, and mutation of RSPO1)
   (b) *Androgen excess* (can be fetal, maternal, or fetoplacental)

Gonadal Dysgenesis

Gonadal dysgenesis is the term used when there is any incomplete or defective formation of the gonads, resulting either from disturbed migration of germ cells or from a disturbance of organization in the fetal genital ridge [91]. There are an enormous number of stages at which the normal developmental processes can fail. Dysgenetic gonads can be associated with an underlying problem with structural or numerical anomalies

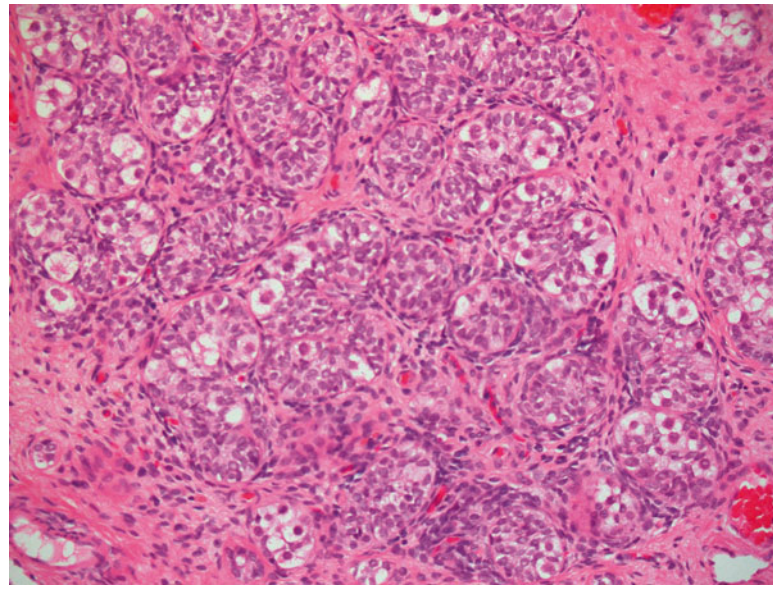

Fig. 14.30 Testis in an individual with 45,X/46XY karyotype. Note prominent immature atypical germ cells, very difficult to distinguish between immaturity and ITGCNU in this situation H&E ×200

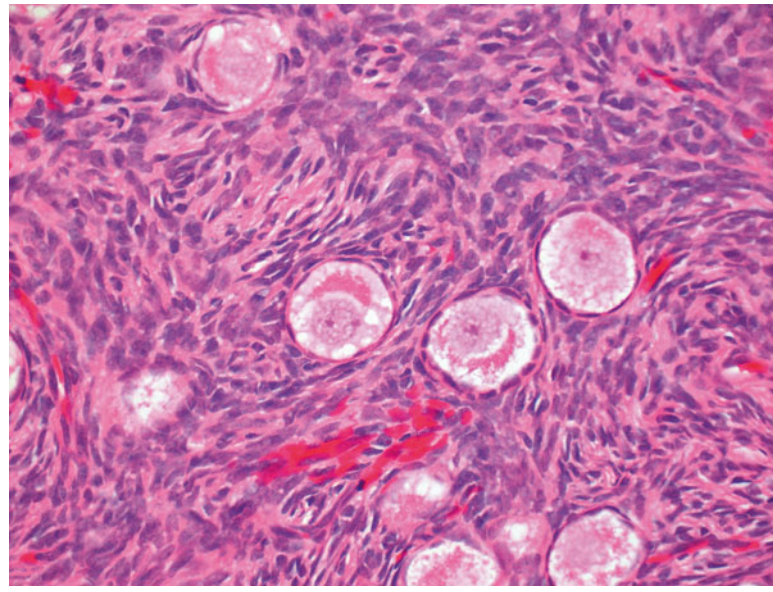

Fig. 14.31 Ovarian tissue in 46,XX individual who also had testicular tissue in the other gonad H&E ×200

of the sex chromosomes, there may be mutations in a gene involved in the formation of the urogenital ridge, or there may be an abnormality of sex determination of the gonad when it is at the bipotential stage.

Histologically there are four patterns of differentiation in dysgenetic gonads:

1. *Testicular differentiation* – defined by the presence of seminiferous tubules (Fig. 14.30).
2. *Ovarian differentiation* – defined as gonadal tissue containing germ cells enclosed in follicular structures (Fig. 14.31).
3. *Streak gonads* – consisting of fibrous stroma devoid of germ cells.
4. *Undifferentiated gonadal tissue* (*UGT)* (Fig. 14.32) – UGT differs from other patterns in that there are germ cells, but these are organized in neither seminiferous tubules nor

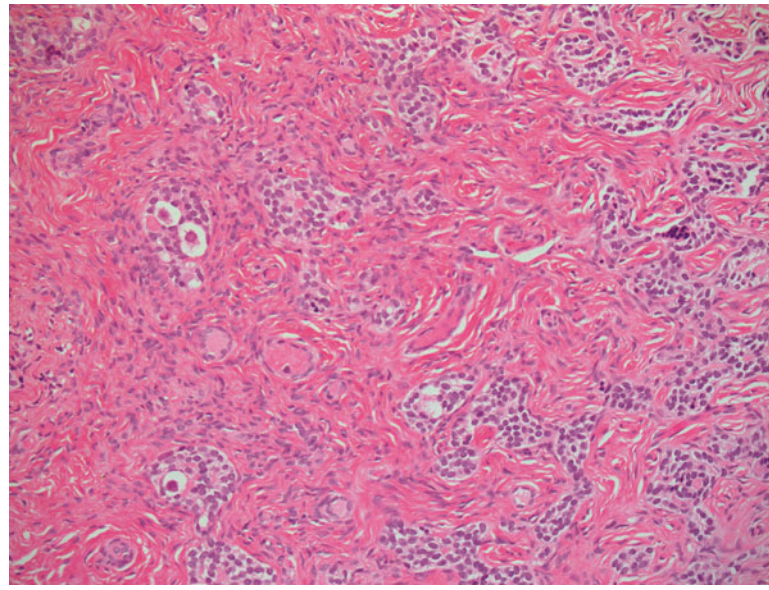

Fig. 14.32 Undifferentiated gonadal tissue, from 46X/46XY individual. Sex cords are not obviously forming seminiferous tubules or follicular structures. Germ cells are present. The morphology here seems suggestive of an early gonadoblastoma. H&E ×200

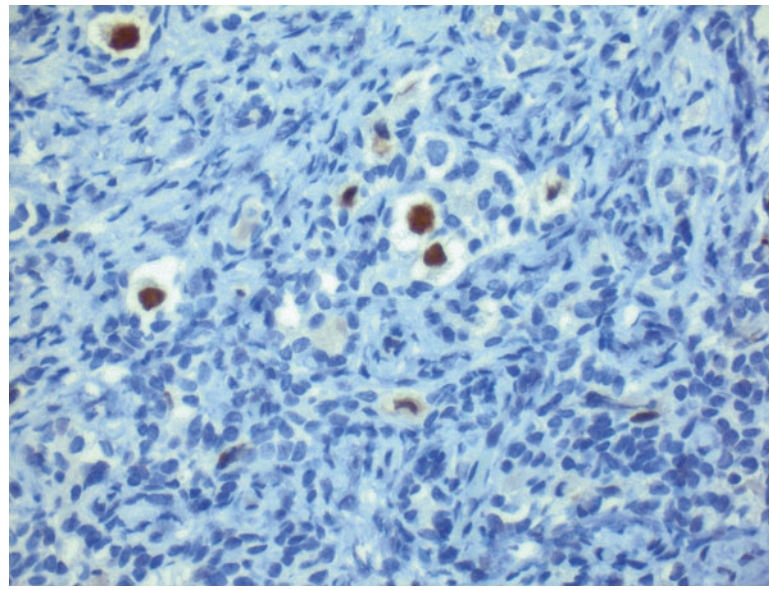

Fig. 14.33 Undifferentiated gonad stained with antibody to OCT3/4. Germ cell nuclei positive ×400

follicular structures but are randomly distributed within the background of stromal cells or aligned in clusters. The germ cells may be in close connection with the sex cord cells, but these structures are not clearly recognizable as Sertoli/granulosa cells. UGT requires markers in order to identify the germ cells that are otherwise easily overlooked [92]. These germ cells are commonly OCT3/4, alkaline phosphatase, and c-KIT positive [7]. OCT3/4 can be particularly helpful in identifying the germ cells in UGT enabling distinction between UGT and streak gonads (Fig. 14.33). UGT is the type of gonad in which gonadoblastoma, described below, can develop. The dysgenetic gonad may also contain both testicular differentiation and UGT patterns within the same gonad [7].

DSD and Germ Cell Tumors

A major clinical problem associated with DSD and the associated dysgenetic gonad is that there is an increased likelihood of developing malignant germ cell tumors [8]. Indeed DSD is one of the major risk factors for the development of the neoplasms in this group.

Though the treatment approach in the past has tended to be early gonadectomy, this radical but safe approach carries with it the complication of infertility. The way in which individuals with DSD are managed has changed radically over the past decade [90], leading to attempt to define more exactly what the risk of neoplasia is in each circumstance and, perhaps, to permit a more conservative approach.

Factors Affecting the Risk of Germ Cell Tumor in DSD

Interestingly, Klinefelter syndrome is the only condition in this group that predisposes to extragonadal germ cell neoplasia (the mediastinum is the common site here [7]). In other conditions, the increased risk is for gonadal tumors. In general, it is only those DSD patients with hypovirilization or gonadal dysgenesis that are at risk of malignant germ cell tumors [7].

For those patients in the "at risk" categories, there are a number of general considerations defining the risk. For example, the anatomical position of the gonad is an important factor, with intra-abdominal gonads conferring a higher risk of neoplasia; this is not unexpected, given the fact that cryptorchidism is known to be a strong risk factor for malignant germ cell tumors in the general Caucasian population [7]. When intra-abdominal gonads develop invasive germ cell tumors in the context of DSD, the histology is usually that of a seminoma/dysgerminoma [7].

Just as in the normal testis, germ cell tumors are associated with a precursor lesion. Depending on the nature of the underlying gonad, this can be ITGCNU or gonadoblastoma (considered in more detail below) – or, possibly, both premalignant lesions may be seen in combination [7].

The Y Chromosome

The risk of developing malignant germ cell tumor in DSD is closely related to the presence of a specific section of the Y chromosome, known as the gonadoblastoma region (GBY) [7]. The area is located close to the centromere. It is not the SRY gene; those 46,XX individuals with a translocation of the SRY gene to an X chromosome or to any other chromosome are 46,XX males, but with no increased risk germ cell neoplasia [7].

The candidate gene for the increased risk is *TSPY* [10]. This seems to be a gene that represses androgen signaling by trapping the androgen receptors in the cytoplasm, so leads to androgen insensitivity in the local environment [10]. Interestingly, it has been shown that a group of gonadoblastomas (by definition occurring in dysgenetic gonads and individuals with DSD) showed positive staining with the corresponding TSPY protein, whereas germ cell tumors occurring in the ovaries of 46,XX women were consistently negative for this [10]. Therefore, it seems that while the germ cell tumors in the ovary and in the dysgenetic gonad have many features in common, the pathways leading to them are likely to be distinct, in at least some points of the process. It seems that it is in the dysgenetic gonad that part of the Y chromosome is essential for the development of a germ cell tumor. It is not a requirement in the normal ovary and therefore not essential for the development of all malignant germ cell tumors.

c-KIT Mutations in Germ Cell Neoplasia in the Dysgenetic Gonad

Though as yet there have been relatively few studies, it might be that *c-KIT* mutations are more important in the etiology of testicular seminoma and ovarian dysgerminoma in the absence of DSD; one study found that *c-KIT* mutations could be identified in these two tumors in non-dysgenetic gonads, but not in gonadoblastomas and associated invasive germ cell tumors in cases of DSD, again suggesting that there may be more than one pathway in the development of germ cell tumors [10].

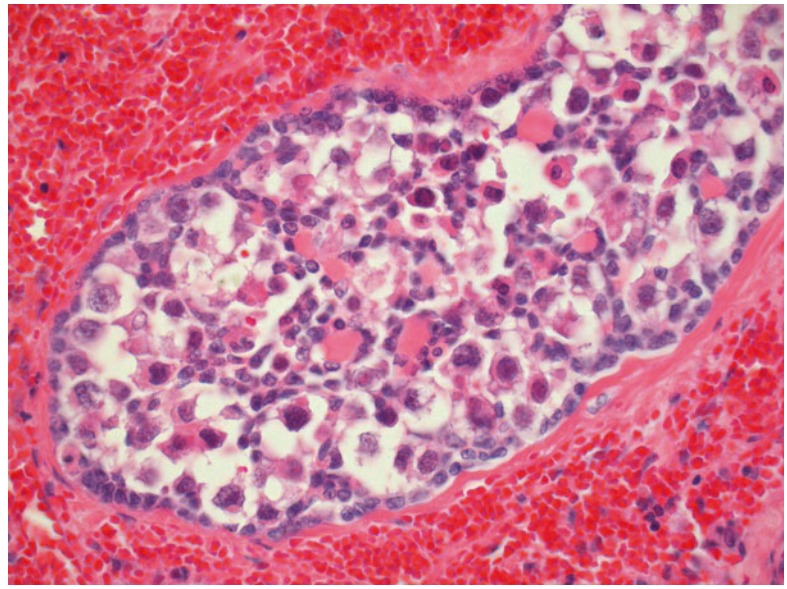

Fig. 14.34 Gonadoblastoma H&E ×200

Gonadoblastoma

Gonadoblastoma is a distinct neoplasm histologically composed of two cell types: large germ cells showing some features of similarity to seminoma cells and small cells resembling Sertoli and granulosa cells [92], but which cannot be regarded as having differentiated into these cells (Fig. 14.34). It probably occurs almost entirely in dysgenetic ovarian tissue. Its presence most probably implies that the underlying gonad must be dysgenetic. This is the premalignant lesion that is, in the undifferentiated dysgenetic gonad, the counterpart of intratubular germ cell neoplasia (ITGCNU) in the testis. As noted above, *TSPY* gene is a possible candidate for the involvement of the Y chromosome in the development of germ cell neoplasia in dysgenetic gonads [3].

There is a suggestion that gonadoblastomas might be more analogous to the ovarian follicle than the testicular seminiferous tubule. While the supporting cells within the seminiferous tubules of ITGCNU express SOX9, indicative of testicular development, the sex cord-stromal cells of gonadoblastomas do not (or express SOX9 very faintly), but they do express FOXL2, suggesting that there is a closer analogy to ovarian sex cord structures such as granulosa cells [8].

It is not infrequently an incidental finding when gonadectomy takes place for DSD. Since it is the preinvasive form of germ cell neoplasia, it may be found in association with an invasive germ cell neoplasm, most commonly dysgerminoma. Commonly there is only a microscopic focus of gonadoblastoma present, though larger tumors do occur, with examples recorded up to 8 cm [59]. In view of its association with DSD, bilateral tumors are common – around 40 % involve both ovaries [59].

Gonadoblastoma is usually easily identifiable on general histological grounds, with its very characteristic morphology. However, if immunocytochemistry is deemed helpful in a given case, OCT3/4 is present in the germ cells of all gonadoblastomas [6]. The supporting cells express inhibin.

Gonadoblastoma itself does not metastasize, but many malignant invasive germ cell tumor developing from it will have metastatic potential.

Risk of GCT Development in Different Types of DSD

There is some data on the risk from various published series, though it is always difficult to extrapolate from these to the individual patient. These are rare disorders, and published series may have a certain bias due to different ways of classifying DSD over the years. Also the

practice of prophylactic gonadectomy in DSD has modified the natural clinical course. The prevalence in untreated individuals may be much higher.

Cools et al. provided one of the most comprehensive meta-analyses of the relevant risks in 2006 [91]. Interestingly other types of gonadal neoplasm are also reported in DSD including sex cord-stromal tumors and epithelial tumors [91]. Traditionally the prevalence of germ cell tumors (either invasive or in situ – either ITGCN or gonadoblastomas) in patients with gonadal dysgenesis is quoted at 30 % [91]. However, Cools et al. point out that this is most probably simplistic and the prevalence can vary widely depending on the particular condition involved. Overall, using the available literature, they estimate that the prevalence in those with the various types of gonadal dysgenesis is 12 % [91]. The following gives an account of the prevalence in several broad types of DSD:

1. Hypervirilization – This group includes the most common forms of DSD, the 46,XX individual with virilized external genitalia as a result of excess exposure to androgens because, for example, of a disorder of adrenal steroid hormone synthesis. As already stated above, they have intrinsically normal ovarian tissue and they are *not* at a risk for the development of germ cell tumors [91].
2. Hypovirilization – In the undervirilization syndromes such as complete androgen insensitivity syndrome (CAIS, previously known as "testicular feminization"), or partial androgen insensitivity syndrome (PAIS), the overall prevalence of germ cell neoplasia is estimated by Cools et al. to be around 2.3 % but is less for CAIS than for PAIS [91]. In the former group, where germ cells are usually lost rapidly from the time of infancy onward, it is less than 1 %. In PAIS, this germ loss is much less rapid and at puberty many PAIS patients have two thirds of their germ cell population [91]. In PAIS and CAIS, tumor prevalence increases after puberty and reaches 33 % by the age of 50 years (many patients have undergone gonadectomy well before this age, so in countries with access to modern medicine, it is rare to encounter a non-gonadectomized patient at this age) [91]. Though there is a quoted prevalence of 5.5 % in infant gonads with these disorders, Cools et al. suggest that this may be an overestimate because of the problems of overdiagnosis of ITGCNU in immature gonads [91], discussed below.
3. DSD with gonadal dysgenesis – Many separate conditions result in incompletely or defectively formed gonads. The literature here is confusing, because of problems of nomenclature and definition and also because of the problems of diagnosis of in situ lesions in immature dysgenetic gonads. Cools et al. consider that germ cell neoplasia – either in situ or invasive – occurs in 15 % of dysgenetic gonads using the data from reported cases [91]. This may be lower for those with ovotesticular DSD. It may be considerably higher for those with 45,X/46XY sex chromosome DSD.
4. DSD with WT1 mutations – Though published studies are few, it does appear that the incidence of germ cell neoplasia in those with WT-1 mutations may be as high as 60 % [91]. This group of conditions includes Frasier syndrome which is characterized by chronic renal failure in early adulthood with focal and segmental glomerulopathy on histological examination, dysgenetic gonads, and (usually) a female phenotype in those with a 46,XY karyotype [10]. Other syndromes resulting from WT-1 mutations include Denys-Drash syndrome. This is characterized by early renal failure (with the histological lesion of diffuse mesangial sclerosis), a high risk of Wilms' tumor, and genital abnormalities in 46,XY individuals, and it has been suggested that there may be some clinical overlap with Frasier syndrome [93]. WAGR syndrome (Wilms' tumor, aniridia, genitourinary anomalies, and mental retardation syndrome) has also been reported in association with gonadoblastoma [94]. However, there are a relatively small number of reported cases in this group, and this does make it difficult to extrapolate in order to predict the risk in an individual patient.

Diagnosis of Early Germ Cell Neoplasia in DSD

In patients with dysgenetic gonads, 92 % of premalignant germ cell lesions are in the form of gonadoblastoma and only 8 % occur as ITGCNU within a dysgenetic testis [91]. In 46,XY hypovirilization syndromes, such as CAIS or PAIS, the gonad involved will always be a testis [91].

As already discussed, gonadoblastoma is usually easily identifiable from its distinctive histopathological features. In the testis of young DSD patients, accurate identification of premalignant germ cell lesions can be difficult. In the adult testis, germ cells do not express OCT3/4, so its presence is a useful indicator of ITGCNU. Other well-known markers for ITGCNU in the adult testis (and that of the older child) include PLAP and c-KIT [95]. However, there is a problem in the immature testis. All of these three markers can persist in infants and young children with DSD in the absence of a premalignant germ cell lesion, since delayed maturation is often an associated feature [8]. A further problem of identification of ITGCNU in this group is that primitive gonocytes and ITGCNU have similar morphological features. The immature germ cells seen in this situation tend to be very large and irregular, with abundant pale cytoplasm and large hyperchromatic nuclei [95]. Thus, in a testis from an infant or young child with a condition in this group of disorders in which there are germ cells with atypical morphology showing nuclear positivity for OCT3/4 (or positivity for other markers such as PLAP or c-KIT), a diagnosis of ITGCNU should be made with caution. Cools et al. [95] propose three criteria that can help distinguish immature germ cells in the testes of hypovirilized DSD patients from those showing features of premalignant transformation. They found that OCT3/4 was a more consistent marker than c-KIT or PLAP [95]. In children up to 1 year of age with hypovirilized DSD, OCT3/4-positive germ cells are an expected finding in accordance with delayed maturation, and positive staining with this marker is insufficient for the diagnosis of ITGCNU [95]. They also noted that the distribution of the OCT3/4-positive cells was different in delayed maturation; in ITGCNU, the abnormal cells were always located on the basal lamina of the seminiferous tubules in one focus within the gonad but tended to be luminal and located throughout the gonad in delayed maturation [91]. They suggest that a diagnosis of ITGCNU should not be made in patients aged less than 1 year and always with considerable thought during early childhood [91, 95].

Other Associations of Ovarian Germ Cell Tumors

Systemic mast cell disease and other hematological conditions are described in germ cell neoplasms, though usually in association with a mediastinal GCT [96]. However, mast cell proliferations have also been seen in the context of an ovarian neoplasm [96]. There is the possibility that this involves the *c-KIT* tyrosine kinase surface receptor.

Autoimmune Phenomenon

There is an association between ovarian teratomas and autoimmune disorders. These include hemolytic anemia, and more recently autoimmune encephalitis associated with antibodies to N-methyl-D aspartate receptor (anti-NMDAR) has been described [21]. This is a clinically severe form of encephalitis, diagnosed by the presence of the relevant antibodies in the CSF in the absence of other causes of encephalitis and other laboratory findings associated with them. The majority of the associated tumors are ovarian teratomas containing neural tissues to which anti-NMDA receptor antibodies develop. There are a few other reported cases associated with teratomas at other sites and with very few non-teratomatous neoplasms [21]. The ovarian teratomas are commonly MCTs, though, relatively speaking, a greater proportion of the much less common immature teratomas are associated with this condition. As discussed above, dysgerminoma has also been reported with

paraneoplastic encephalitis associated with anti-Ma2 antibodies, in which the relevant antigen is shared by the tumor cells and by normal human brain [66, 97].

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