Germ Cell Tumors of Infancy and Childhood

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Introduction

Testicular tumors in the prepubertal period are rare. The annual incidence is estimated to range from 0.5 to 2.0 per 100,000 boys, accounting for 1–2 % of all solid pediatric tumors although it is increasing [1, 2]. The age distribution of prepubertal testicular tumors is bimodal, with one peak occurring in the first 2 years of life and a second occurring in young adulthood. The frequency of the distinct testicular tumors in pediatric age varies among series, depending on whether the adolescence period is included or not. Germ cell tumors account for 71 % (yolk sac tumors [YSTs], 49 %; teratomas, 13 %; seminoma and mixed germ cell tumors, 9 %), and gonadal stromal tumors account for 29 % of prepubertal testicular tumors [3]. About 70–75 % of prepubertal tumors are benign [4].

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Germ Cell Tumors (GCTs)

There are several differences between GCTs occurring in children and those of adults from the histopathologic, molecular biology and biologic behavior points of view. Most are pure YSTs or teratomas [5, 6]. GCTs account for 71 % of testicular tumors in childhood versus 96 % in adulthood. Cryptorchidism does not play an important role as an underlying factor, as fewer than 40 cases have been reported in undescended testes [7]. By contrast, ethnicity is important: prepubertal tumors are more frequent in Asian-Pacific Islanders than in white men, while the incidence is lowest among blacks [8]. The peritumoral parenchyma rarely shows germ cell neoplasia in situ (GCNiS) also known as carcinoma in situ (CIS), which suggests that GCTs in prepubertal boys have a different origin from those in adolescents and adults [9].

Moreover, it should be emphasized that the cytokinetic profile for the same histologic tumor type is different. YSTs in prepubertal children show chromosome 1 imbalances, loss of 6q, and chromosome 20 anomalies [10–13], while isochromosome 12p is present in 80 % of adult cases and enlargement of 12p segments is found in the remaining adult cases. Both YSTs and teratomas in childhood are diploid [14].

GCTs appearing in pubertal age or immediately afterward do not have any morphologic,

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immunohistochemical, or cytogenetic differences with respect to adult GCTs [15–18].

Yolk Sac Tumors, Prepubertal Type

This is the most common testicular tumor in infancy and childhood, accounting for about 70 % of pediatric GCTs [19]. The age of onset varies from birth to 9 years of age, with a peak incidence at 18 months. Most YSTs in children are pure, unlike those in adults [20]. One case associated to microlithiasis has been reported [21].

Clinical Features

The most common presentation (>90 %) is of a painless scrotal mass. A hydrocele is associated with a testicular tumor in 15–50 % of cases [22], and in 6 % of patients, metastasis has been reported as the first finding [23]. One percent of YSTs are bilateral, while in unilateral cases, there is a slight predilection for the right testis. The diameter ranges between 2 and 4 cm. Ninety percent of patients have significant elevation of serum alpha-fetoprotein (AFP) [24]; this should, however, be interpreted with caution as such elevation can overlap with normal levels. Regarding AFP levels in infants, it should be taken into account that 50,000 ng/mL is considered normal at birth; the level then decreases progressively to 10,000 ng/mL at 2 weeks, 300 ng/mL at 2 months, and 12 ng/mL at 6 months [25].

Pathologic Features

The macroscopic appearance of YST in children is a solid, white to gray mass replacing most of the testicular parenchyma. The cut surface is microcystic, myxoid, or gelatinous. Small foci of necrosis and hemorrhage can be observed (Fig. 4.1).

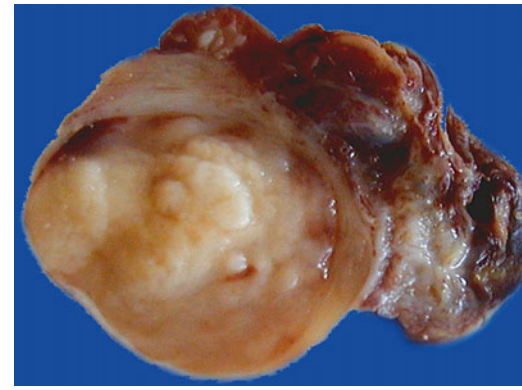

Fig. 4.1 Yolk sac tumor. The whitish multinodular tumor replaces two-thirds of the testicular parenchyma

Numerous microscopic patterns including microcystic, endodermal sinus, papillary, solid, glandular, myxomatous, sarcomatoid, macrocystic, polyvesicular vitelline, hepatoid, enteric, and parietal patterns can be found in YSTs [20, 26–28].

The *microcystic pattern* is the most common and is characterized by a reticular pattern that recalls a disorganized spider web of cells with large intracellular vacuoles (Fig. 4.2). The cytoplasm is reduced to a thin peripheral ring resembling false lipoblasts, as the vacuoles do not contain lipid. Cells can also be arranged around variably sized small cysts. In this case the cells are small, benign in appearance, and with numerous hyaline globules.

The *glandular pattern* is defined by the presence of glandular or tubular structures, simple or complex, and contiguous to other patterns such as microcystic, myxomatous, solid, or polyvesicular vitelline. Glands may present intestinal differentiation (enteric pattern). In other cases the glands show subnuclear vacuoles resembling secretory endometrium. These glands can be distinguished from teratomatous glands by the lack of a smooth muscle component and the absence of other teratomatous elements.

The *myxomatous pattern* consists of a proliferation of spindle or stellate cells dispersed in a mucopolysaccharide-rich stroma. This pattern usually has a prominent vascular network described as "angioblastic mesenchyme" [27]. The cells resembling extraembryonic mesenchyme are pluripotential, with the capacity to form differentiated tissues such as the skeletal muscle, cartilage, or bone. The differential diagnosis with teratoma is established by the absence of other surrounding teratomatous elements.

The *endodermal sinus pattern* consists of a central vessel surrounded by tumor cells, contained within a cystic space lined with flattened tumor cells. The papillary structures are termed glomeruloid or Schiller-Duval bodies

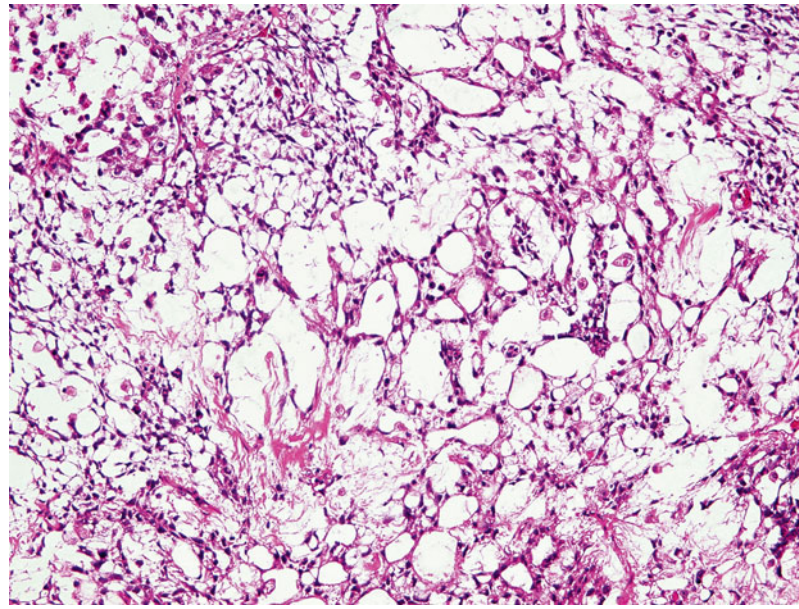

Fig. 4.2 Microcystic pattern of yolk sac tumor showing cells with a large vacuole in the cytoplasm and microcysts created by cords of cells surrounding the extracellular space

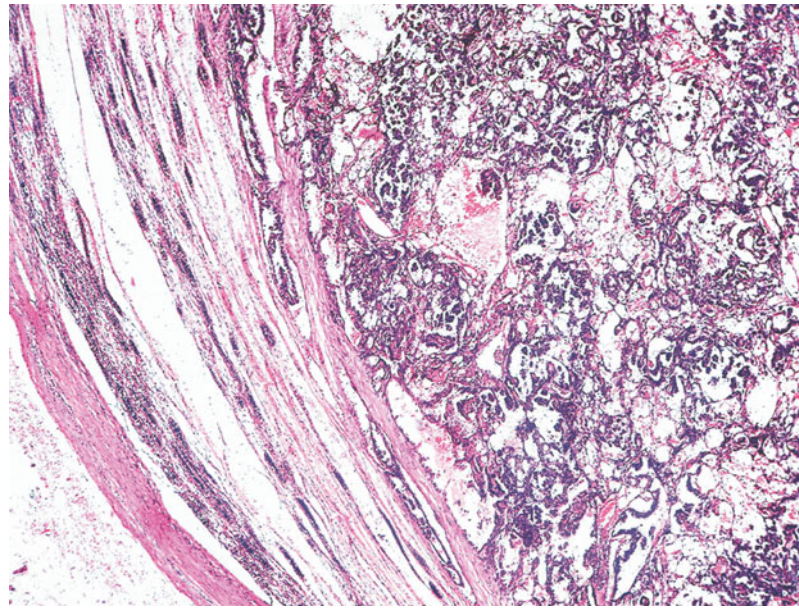

Fig. 4.3 Endodermal sinus pattern of yolk sac tumor showing numerous cystic spaces with glomeruloid structures inside that are formed by tumor cells

because they resemble the endodermal sinus of the placenta of the rat and are responsible for the name by which this tumor was previously known, endodermal sinus tumor. These bodies, although not very numerous, are characteristic of YST (Figs. 4.3 and 4.4).

The *papillary pattern* is recognized by the presence of numerous small papillae, with or without a fibrovascular core, that project into cystic spaces. The cells are cubical or low columnar with hobnail nuclei, resulting in a jigsaw configuration.

The *solid pattern* consists of sheets of uniform cells with clear cytoplasm and well-defined borders. Cells show marked pleomorphism and atypia (Fig. 4.5). The solid pattern of YST is almost always

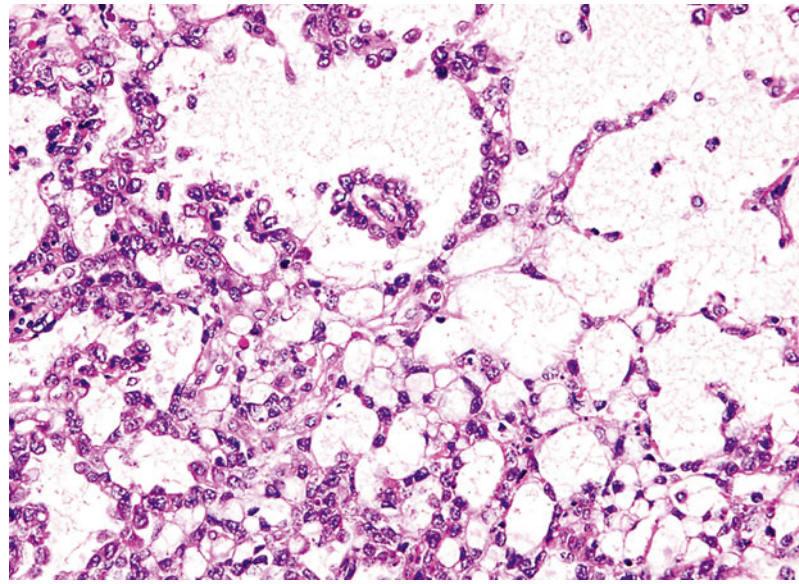

Fig. 4.4 Schiller-Duval body in a yolk sac tumor with microcystic pattern

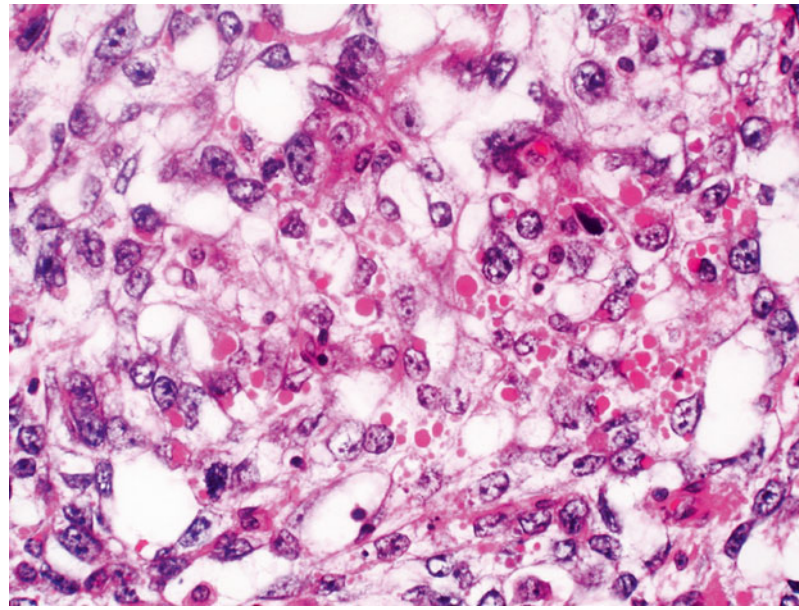

Fig. 4.5 Solid and microcystic patterns of a yolk sac tumor. Numerous eosinophilic bodies can be observed in the cytoplasm of tumor cells

associated with other patterns, most commonly microcystic/reticular (75 %), glandular (35 %), and myxoid (25 %). This tumor may resemble a seminoma that, although rarely, can present in late childhood and puberty, but fibrous septa and a lymphoid component are absent in YST. Moreover, the immunophenotype is different [29].

The *sarcomatoid pattern* consists of a spindle cell proliferation commonly inside a microcystic pattern.

The *macrocystic pattern* usually arises from the coalescence of microcystic spaces to form a large cyst. The cells lining the cysts are flattened and show mild atypia.

The *polyvesicular vitelline pattern* is characterized by the presence of cysts with a central constriction producing two vesicles that remain connected, dispersed in a myxoid stroma (Fig. 4.6). The name refers to the image observed when the secondary yolk sac is formed in a

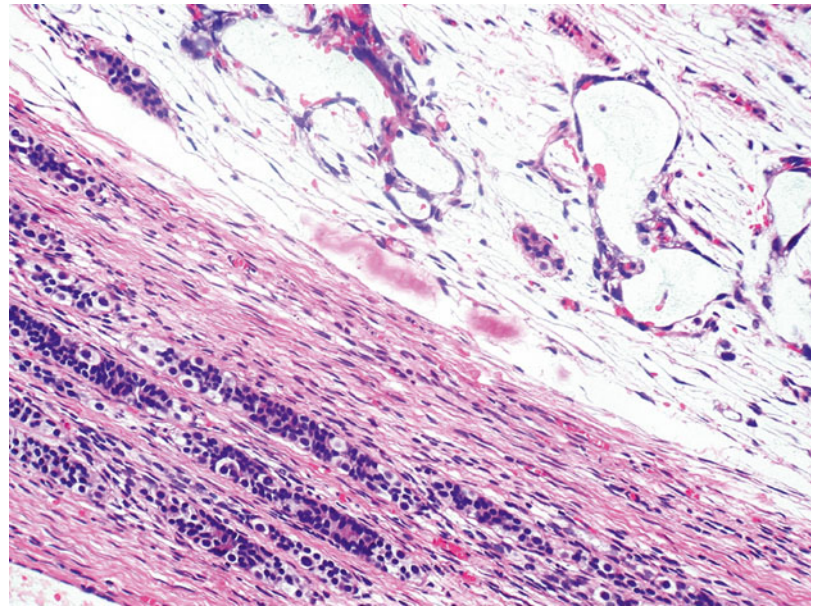

Fig. 4.6 Polyvesicular vitelline pattern of yolk sac tumor. Vesicle-like structures with a partial constriction lined with flattened epithelium in a myxoid stroma

normal embryo. The epithelium lining the second vesicle is commonly columnar, which contrasts with the flattened epithelium of the original cavity. The cells frequently show hyaline globules. The occasional presence of glands with mucous cells or cells with brush borders is interpreted as a differentiation of this second cavity. There is an established parallelism with gastrointestinal differentiation from the secondary yolk sac in the normal embryo.

The *hepatoid pattern* is also interpreted as a differentiation from yolk sac. It is characterized by clusters of polygonal cells arranged in sheets, nests, or cords. The cells have large, round, vesicular nuclei with prominent nucleoli. Atypia and mitosis are common. The cytoplasm may be clear, slightly amphophilic or basophilic, and may contain hyaline globules. This pattern is more common in adult tumors than in children and is almost always focal.

The *parietal pattern* has extensive and irregular deposits of extracellular basement membrane. Tumor cells are arranged surrounding or inside these masses. This pattern is named after the resemblance of the thick basement membrane (Reichert's membrane) of the parietal layer of the vitelline sac of rodents.

Most times YST is formed by different patterns in a myxoid or fibrous stroma or a highly cellular mesenchyme. The most common patterns are microcystic, solid, and myxomatous. The polyvesicular vitelline, sarcomatoid, and parietal patterns are rare. The glandular and parietal patterns are most often seen after chemotherapy.

Immunohistochemistry

Most tumor cells show positivity for cytokeratins and glypican-3 by immunohistochemistry, while staining for OCT3/4 is uniformly negative. Placental-like alkaline phosphatase (PLAP) positivity is found in 39–85 % of tumors [26]. AFP is focally observed in most tumors except for those with a hepatoid pattern that is diffuse, with or without a relationship with hyaline globules. Fifty percent of tumors show positivity for alpha-1 antitrypsin, while vimentin is positive in YSTs with myxomatous and sarcomatoid patterns. Laminin is characteristically positive in the parietal pattern. The enteric glands express carcinoembryonic antigen [28].

Biologic Behavior

YSTs in children have a much better prognosis than those in adults [30]. The prognosis of pediatric YST is correlated with age, with slower growth rates and lower grades of malignancy observed in younger children. In children younger

than 5 years, the incidence of metastasis is no higher than 5 %, which increases to 20 % in adults [31]. The most common route of dissemination is hematogenous and the lung is the most common metastatic location [22]. In stage I YSTs, orchiectomy is sufficient, or even testis-sparing tumor excision and follow-up with chest and abdominal imaging and serum AFP measurement, as these tumors are not associated with GCNiS in the peritumoral parenchyma. Survival rates in stage I are higher than 80 % [32, 33]. In case of recurrence or metastasis, patients can be successfully treated with platinum-based chemotherapy (etoposide, carboplatin, and bleomycin) [34–36].

Teratoma, Prepubertal Type

Teratoma is the second most common testicular GCT in pediatric age according to the Prepubertal Testicular Tumor Registry of the American Academy of Pediatrics [37]. However, there is reason to believe that its incidence has been underestimated and that the real number is not accounted for in large series as not all cases are included because of their benign behavior. The incidence of teratomas may actually be higher than that of YSTs [38–40]. The median age at presentation is 13 months. Some cases have been reported in the neonatal period [41], and teratomas are unusual in children older than 4 years of age. Testicular teratomas in children are different from teratomas in adults in many aspects. Teratoma is not associated with other forms of GCT and bilateralism is exceptional in children [42]. A wide variety of congenital malformations such as spina bifida, retrocaval ureter, hemihypertrophy, and hernia can be associated with testicular teratomas. A total of 30 cases have been reported of teratomas originating in intra-abdominal undescended testes [43].

Clinical Features

Most patients with testicular teratomas present with a testicular mass detected during clinical exploration. Some teratomas contain large cysts filled with mucus or fluid that can be misdiagnosed as hydrocele [44] or may contain simple cysts [45], delaying the diagnosis by 6 months on average [46]. Ultrasonography is a useful method to identify a well-delimited, partially cystic intratesticular lesion. In teratomas occurring at prepubertal age, serum markers such as beta-HCG and AFP show normal levels except for teratomas with intestinal glands, which show moderate elevation of AFP.

Pathologic Features

Macroscopically, teratomas are cystic tumors with solid areas. The cysts may measure up to 1 cm in diameter and contain mucous, watery fluid or keratin. Solid areas may contain cartilage or bone.

Microscopically, teratomas are neoplasms that can show endoderm-, mesoderm-, or ectoderm-derived tissues in a disorganized or organoid arrangement. Like teratomas, postpubertal type childhood teratomas can contain mature and immature tissues. Mature teratomas contain cysts lined with squamous, ciliated respiratory-type or intestinal-type epithelium next to smooth muscle, cartilage, bone trabeculae, neuroglia, choroid plexus, and occasionally pigmented epithelium and pancreatic tissue (Figs. 4.7 and 4.8). Immature teratomas contain different types of immature tissues such as neuroepithelium (small cells in tubular or rosette arrangement) and blastema (nodular collections of cells with hyperchromatic nuclei and scant cytoplasm with or without tubular structures inside). The seminiferous tubules in the peripheral parenchyma show hypertrophic or multinucleated germ cells.

Dermoid cyst is a variant of prepubertal, type teratoma, uncommonly present in childhood [47, 48]. Macroscopically, it consists of one large cavity filled with cellular detritus and hair. Microscopically, all epidermal and dermal components are represented. Sebaceous glands are usually numerous (Figs. 4.9 and 4.10). Some authors accept as dermoid cysts tumors containing in their walls other tissues such as smooth muscle, adipose tissue, cartilage, bone, thyroid, salivary gland, gastrointestinal tissue, respiratory-type epithelium, and mature neural tissue [49].

Epidermoid cyst is defined as an encapsulated cyst lined with keratinized stratified flat

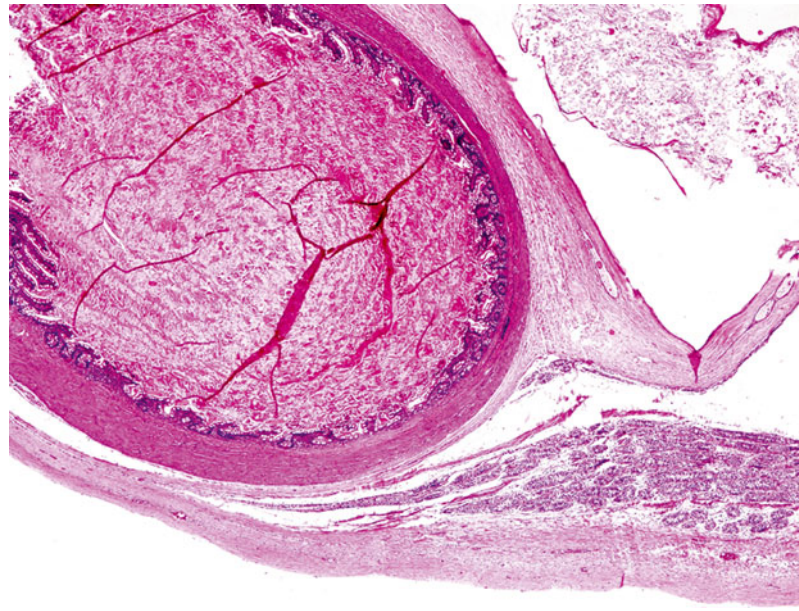

Fig. 4.7 Cystic organoid teratoma in a 10-year-old boy. The spherical formation resembles a complete intestinal wall. The remaining content of the cyst is made up of abundant keratin material

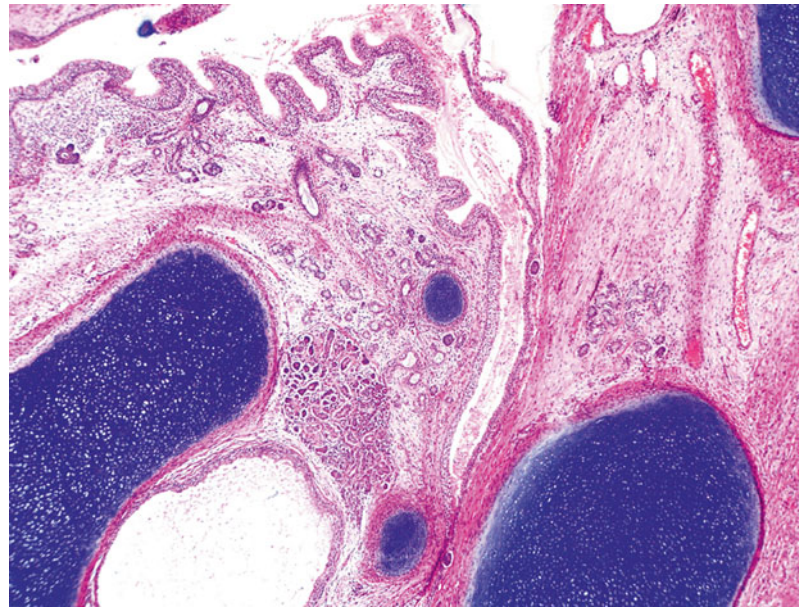

Fig. 4.8 Mature teratoma with islands of hyaline cartilage and cysts lined with nonkeratinized epithelium and glandular structures

epithelium without skin adnexal structures [50]. The keratin masses that fill the cyst produce a characteristic ultrasonographic image resembling onion layers or a dartboard. Most authors consider this lesion as nontumoral and some consider it a as monodermal teratoma. Epidermoid cysts are rare in the pediatric age [51].

Testicular primary carcinoid tumors have been reported as monodermal teratomas [52].

Biologic Behavior

The biologic behavior of teratomas in prepubertal age is benign, independent of their maturity or immaturity (unlike teratomas in adulthood). The nonmalignant behavior justifies enucleating or testis-sparing surgery in cases of bilateral tumors or unique testis [53]. This biologic behavior is related to the absence in childhood (except in 1 reported case [54]) of GCNiS in the

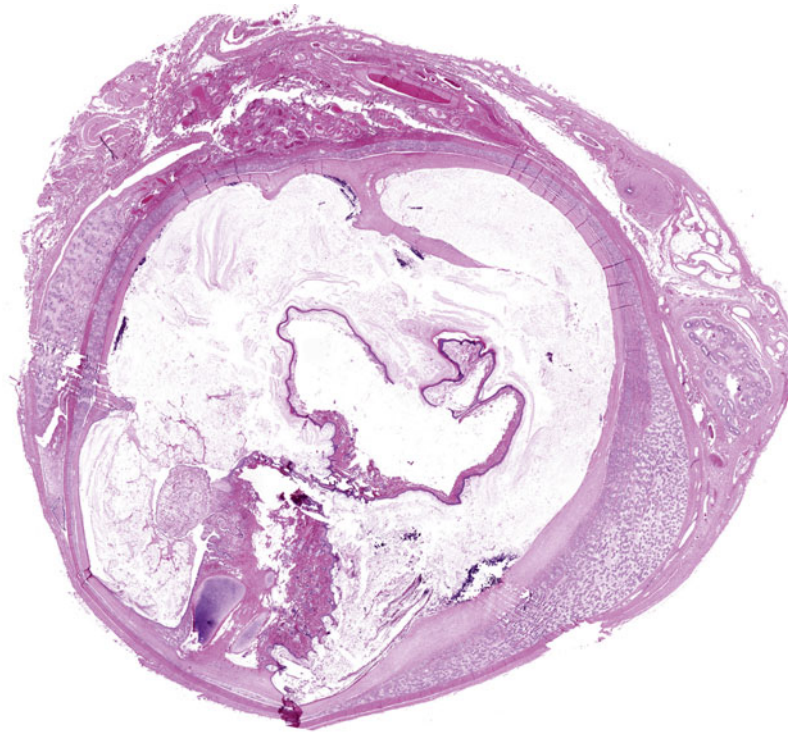

Fig. 4.9 Dermoid cyst. The encapsulated cyst comprises the testicular parenchyma

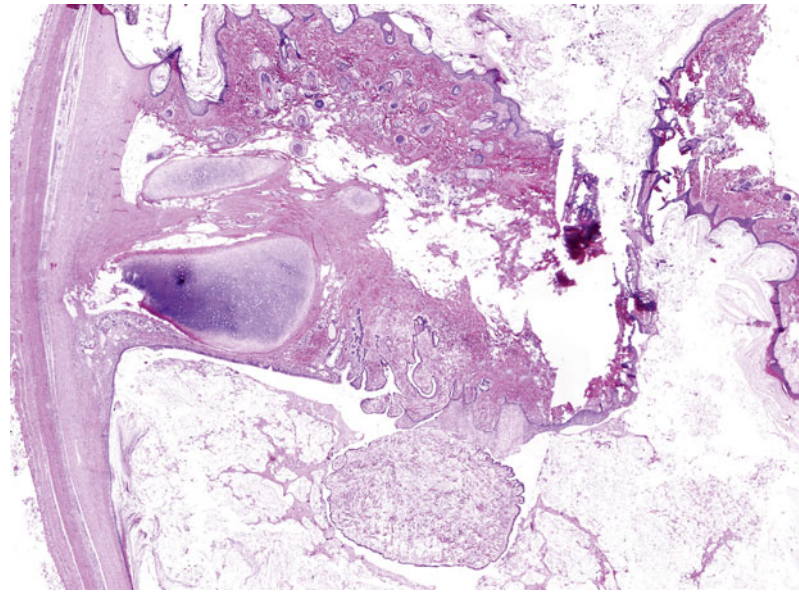

Fig. 4.10 Dermoid cyst partially lined with skin and adnexal pilosebaceous structures as well as respiratory-type epithelium in the septa. Note several islands of cartilage as another component

residual parenchyma, as previously mentioned [55]. In adolescents, orchiectomy and evaluation with the same protocols as used in adult GCTs are recommended. A small percentage of pediatric teratomas have a malignant somatic component, as is frequently observed in adult testis. In these unusual cases, treatment should be completed with chemotherapy or surgery depending on the nature of the malignant component [56].

Other Germ Cell Tumors

Some cases of embryonal carcinoma [34], seminoma [57], and mixed GCT [58] have been reported in childhood and more frequently adolescence. These tumors have a very aggressive behavior, presenting as metastatic disease at diagnosis [59, 60]. After puberty, these tumors are similar to those in adults [61].

Gonadoblastoma

Gonadoblastoma is a mixed tumor composed of a mixture of germ cells and cells resembling Sertoli/granulosa cells arranged in a preferably nodular pattern in an ovarian-type stroma [62]. It characteristically arises in dysgenetic gonads in patients with disorders in sexual differentiation who have either the whole or a part of the Y chromosome in their genome. The most common clinical entities where gonadoblastoma develops are 46XY pure gonadal dysgenesis (patients with bilateral streak gonads) [63–65], mixed gonadal dysgenesis (streak gonad on one side and testicular dysgenesis in the contralateral testis), dysgenetic male pseudohermaphroditism (both gonads with testicular dysgenesis features), ovotesticular disorder [66], patients with Turner syndrome and Y chromosome residual material [67, 68], and androgen receptor insensitivity. Exceptionally, gonadoblastoma has been observed in a normal testis [69].

Eighty percent of patients with gonadoblastomas are phenotypically female, and 20 % are phenotypically male [70]. The *TSPY1* gene (which encodes a testis-specific protein) located at the GBY locus in the centromeric portion of the short arm of the Y chromosome is involved in the genesis of most gonadoblastomas [71, 72]. Gonadoblastoma is assumed to arise from OCT3/4-positive surviving germ cells in undifferentiated gonadal tissue of dysgenetic gonads [73].

The size varies from microscopic to 8 cm in diameter [74]. The tumors are solid and gray or yellow in color. Most gonadoblastomas arise from streak gonads. The remainder arise from testes with a thin and poorly collagenized tunica albuginea resembling the ovarian cortex, with irregular cellular cords or tubules that go through the albuginea to reach the testicular surface [75]. One-third of patients have bilateral gonadoblastomas.

Gonadoblastoma cells are arranged in nests or cords and less frequently in a diffuse pattern; the same tumor can show different patterns. Gonadoblastoma is composed of two types of cells. The first type is germ cells appearing like gonocytes or with seminoma-like morphology because of their large size, voluminous nuclei with one or two nucleoli, and pale cytoplasm. Mitosis can also be seen. The second type of cells is smaller cells with ovoid hyperchromatic nuclei and Charcot-Böttcher filaments in their cytoplasm like Sertoli cells that are admixed with germ cells. Cells with Sertoli features are arranged as an outer palisade in the nodules and around eosinophilic bodies or hyaline nodules that are dispersed by the tumor and formed by basement membrane material (Fig. 4.11). These tumors show prominent calcifications that arise from hyaline bodies. Leydig cells can be observed in the stroma among cords or nests.

Immunohistochemistry

Gonocyte-like cells express OCT3/4, PLAP (Fig. 4.12), c-kit, TSPY, and D2-40 [76], while Sertoli/granulosa cells show inhibin in their cytoplasm and SOX9 and more commonly FOXL2 in the nuclei [77]. Leydig cells are inhibin and calretinin positive.

Gonadoblastoma is considered to be an in situ neoplasia precursor of infiltrating tumors like germ cell neoplasia, in situ. The level of testicularization of the gonad will determine the histologic composition of the precursor lesion, either gonadoblastoma or GCNiS [78]. When germ cells proliferate, they actively destroy the nodular arrangement of the tumor and infiltrate the gonad, giving rise to any type of GCT, although the most common are seminoma and dysgerminoma [79] (Fig. 4.13). Gonadectomy is recommended in all cases because of the infiltrating ability of the tumor.

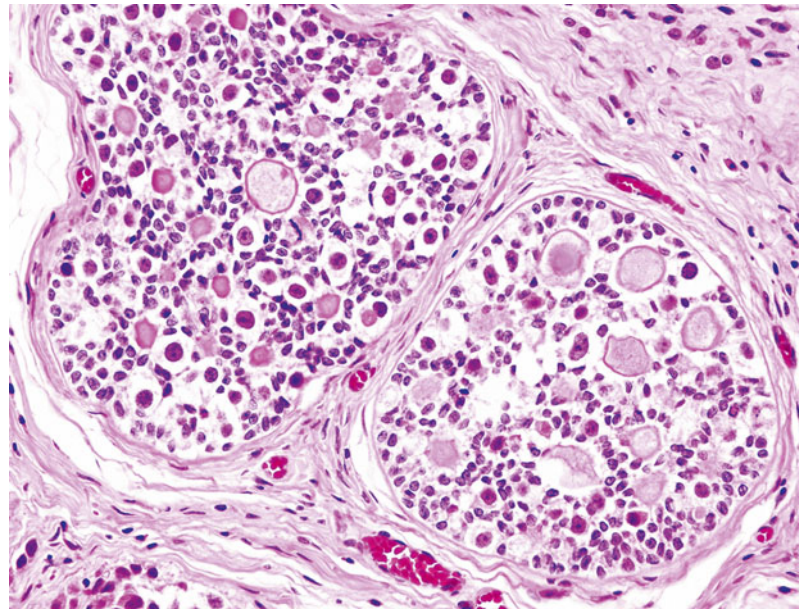

Fig. 4.11 Gonadoblastoma. Nests made up of two cell types. The smaller cells have hyperchromatic nuclei and are arranged above the basement membrane and surrounding the abundant eosinophilic matrix. The larger cells have clear cytoplasm and prominent nucleoli

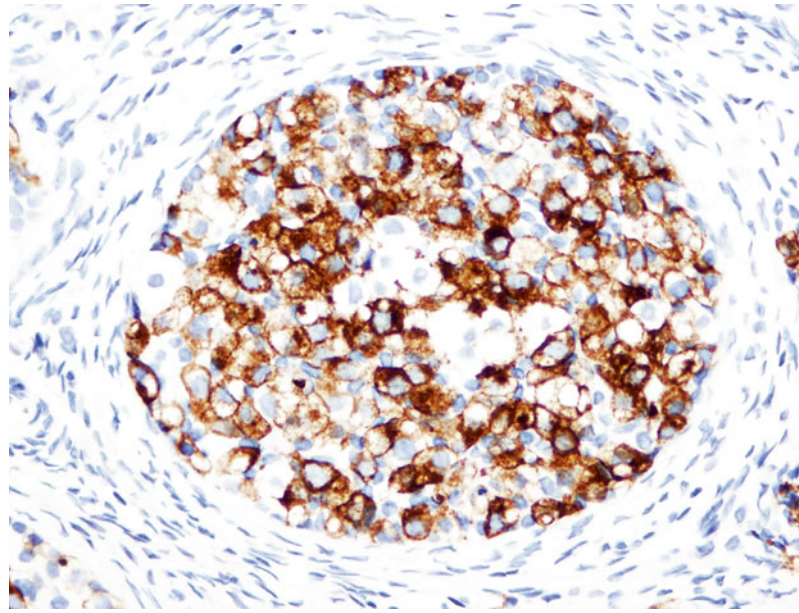

Fig. 4.12 Gonocyte-like cells of a gonadoblastoma show PLAP membranous immunostaining

The differential diagnosis of gonadoblastoma in childhood includes fetal gonadoblastoid testicular dysplasia (abnormally differentiated testicular parenchyma that is associated with Walker-Warburg syndrome) as well as ureter malformations and renal cystic dysplasia. The peripheral testicular parenchyma shows a crescent-like zone with spherical or irregular solid nests, cords, and malformed tubules that are embedded in an undifferentiated stroma. Each structure is composed of three cell types: cells with vesicular nuclei and vacuolated cytoplasm, cells with hyperchromatic nuclei, and germ cell-like cells. However, gonocyte or dysgerminoma-like cells are absent unlike gonadoblastomas.

In postpubertal patients, Sertoli cell nodules colonized by GCNiS cells may be misinterpreted

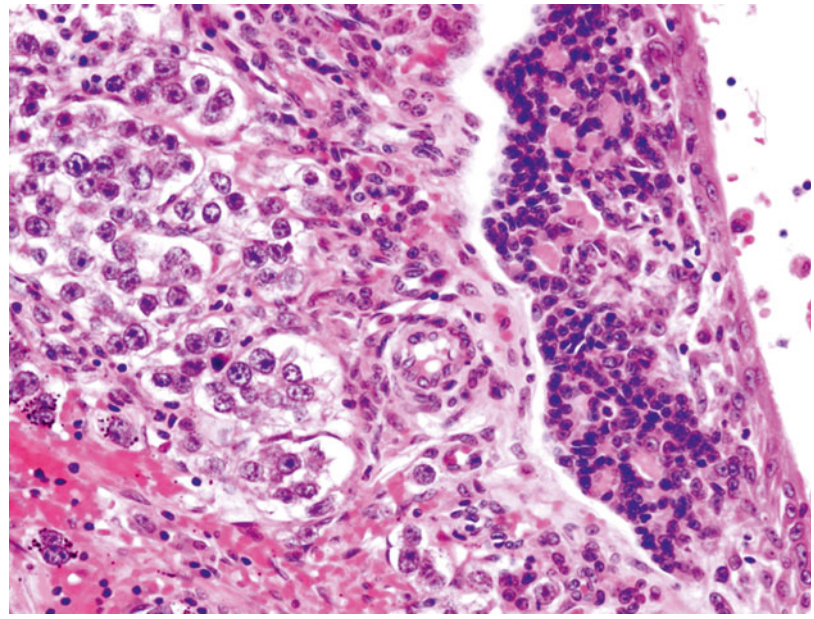

Fig. 4.13 Dysgerminoma originated from gonadoblastoma. Remnants of gonadoblastoma can be recognized with Sertoli/granulosa cells and Call-Exner bodies

as gonadoblastoma. Sertoli cell nodules are common in cryptorchid testes of postpubertal patients without any intersexual condition. Sertoli cell nodules colonized by GCNiS cells are usually in the vicinity of seminoma or seminiferous tubules with GCNiS. Sertoli/granulosa cells express both SOX9 and FOXL2, and this is evidence of their incomplete differentiation. In contrast, Sertoli cell nodules with GCNiS show diffuse and intense positivity for SOX9 and SF-1 and absence of immunoexpression for FOXL2 as evidence of their complete differentiation [80].

Pediatric-Type GCTs (Type I) in Adults

Several types of GCNiS, varying from mature teratomas to monophyletic immature teratomas to YSTs, have been reported in adults that, except for the patient's age, have all the characteristics of pediatric GCTs (type I). These tumors differ from adult GCTs in the following features [81]:

- Absence of GCNiS in the peripheral parenchyma confirmed by OCT3/4 and PLAP.
- Absence of testicular scar or burned-out tumor.
- Absence of inflammatory infiltrates; minimal infiltrates or plasma cell inflammatory infiltrates are accepted.
- Demonstration by FISH of two centromeres in chromosome 12 and absence of 12p excess.
- Complete spermatogenesis.

The behavior of this group of tumors is benign, and the recommended treatment should be orchiectomy or even testis-sparing surgery.

The origin of type I GCT in adults is probably a germ cell that is directly reprogrammed to pluripotentiality without going through a GCNiS stage. Like their pediatric counterparts, these tumors probably originate in the neonatal period or some years later, presenting clinical symptoms in puberty or adulthood [82].

Germ Cell Neoplasia In Situ (GCNiS)

Germ cell neoplasia in situ presumably originates during fetal life from gonocytes that do not differentiate properly and maintain their original immature and multipotent features. Morphologic, immunohistochemistry, and genetic profile similarities support this hypothesis [83–85]. The microscopic morphology of GCNiS in childhood

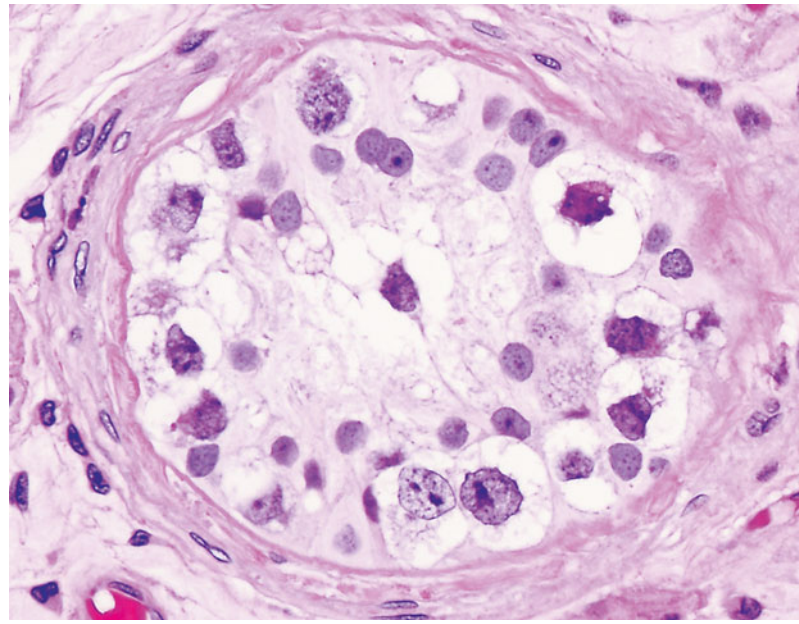

Fig. 4.14 GCNiS cells above the basement membrane displacing Sertoli cells toward the lumen of the tubule. The cells are large and show one or two prominent nucleoli

is different from GCNiS in adults. Before making a diagnosis of GCNiS in infancy or childhood, it is important that one is familiar with the following features: (a) GCNiS features in adult testis, (b) proliferation and differentiation of fetal germ cells, (c) the most useful criteria in the differential diagnosis between a delay in gonocyte maturation and GCNiS in postnatal life, and (d) knowledge of situations with atypical-looking germ cells but without enough criteria to establish a diagnosis of GCNiS.

GCNiS Features in Adult Testis

Germ cell neoplasia in situ is the preinvasive phase of all types of GCTs except for pediatric GCTs and spermatocytic tumor. It is usually found in groups of small seminiferous tubules devoid of spermatogenesis that probably belong to the same lobule. These small tubules are often separated by larger tubules with complete spermatogenesis. The tumor cells are typically confined to the basal compartment of the seminiferous tubule, displacing Sertoli cells luminally. GCNiS cells are large cells with clear cytoplasm because of the high glycogen content and a sharply defined plasma membrane. The nuclei are large, spherical, and hyperchromatic, often with one or two prominent nucleoli and coarse chromatin (Fig. 4.14). Mitosis can be observed. Sertoli cells commonly show spherical instead of the normal triangular nuclei. The nucleoli are central but small, suggesting incomplete maturation [83]. Sertoli cell dysfunction is probably established in fetal life, leading to a defect in the mechanisms of interaction between Sertoli and germ cells and a consequent defect in gonocyte maturation. In 50 % of cases, GCNiS cells acquire infiltrating capacity after 5 years, and a GCT develops [86]. GCNiS cell transformation into seminoma is slower than transformation into a nonseminomatous tumor, which is related to the more aggressive behavior of the latter.

The classical immunohistochemical markers of GCNiS are the transcription factor OCT3/4 [87, 88] and PLAP [89] (Fig. 4.15). GCNiS cells are also positive for the AP2 transcription factor AP2γ [90], podoplanin [91, 92], and c-kit [93]. OCT3/4 is expressed in the nuclei, whereas PLAP shows membranous expression. Most cells that express OCT3/4 also express PLAP. The average nuclear DNA content of CIS cells is about 4C.

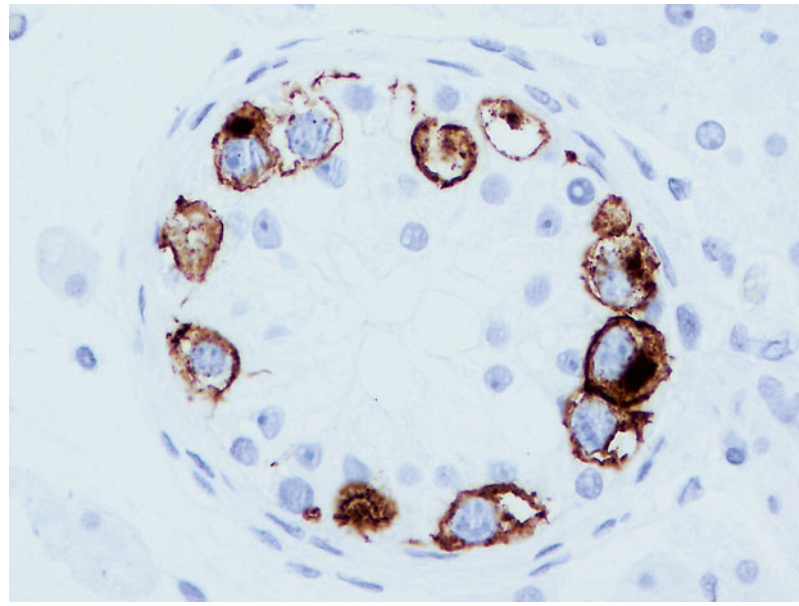

Fig. 4.15 PLAP immunoexpression in the membrane of GCNiS cells

Proliferation and Differentiation of Fetal Germ Cells

Three different morphologic and immunohistochemical types of germ cells have been identified in the testis during the fetal period; these types represent three different stages in the differentiation from primordial germ cell to spermatogonium. One characteristic feature of human fetal testis is the concurrence during several months of the three cell types as opposed to the homogeneous population of gonocytes in other mammalian species.

Gonocytes

Once the primordial germ cells in the genital ridge are surrounded by cells deriving from the coelomic epithelium (pre-Sertoli cells), they transform into gonocytes: gonocytes are identified in the center of the seminiferous cords separated from the basement membrane by Sertoli cells. Gonocytes are large cells that have spherical euchromatic nuclei with one or two nucleoli [94, 95]. The gonocyte immunophenotype is OCT3/4, c-kit, and PLAP positive and melanoma-associated antigen 4 (MAGEA4) negative [96–98].

Intermediate Cells

Intermediate cells (transitional gonocytes) are morphologically similar to gonocytes, although they differ in their distribution in the seminiferous cords and in their immunophenotype. Intermediate cells are located in the periphery of the seminiferous cords in contact with the basement membrane. Their immunophenotype is negative for both c-kit and MAGEA4, whereas they show weak or negative staining for OCT4 and positive staining for proliferating cell nuclear antigen (PCNA), indicating active proliferation. It has been suggested that when these cells get in contact with the basement membrane, they lose their pluripotency and start to differentiate.

Fetal Spermatogonia

Fetal spermatogonia (or pre-spermatogonia) are grouped at the periphery of the seminiferous cords above the basement membrane. They are the germ stem cells of the adult testis. Immunohistochemistry shows MAGEA4 staining, and the cells are negative for c-kit and PCNA.

At the tenth week of gestation, most germ cells are gonocytes; at approximately the 15th week, many intermediate cells are present together with gonocytes, and fetal spermatogonia

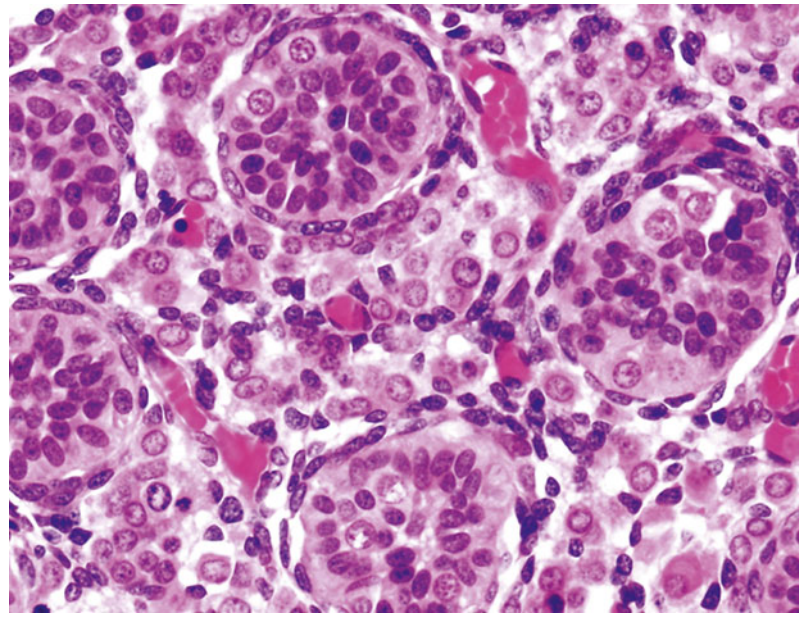

Fig. 4.16 Fetal testis (30 weeks of gestation). Most germ cells are pre-spermatogonia located above the basement membrane

can be observed for the first time. From the 22nd week onward, most germ cells are fetal spermatogonia. Some gonocytes persist even after birth. Most of them differentiate into spermatogonia between 30 and 90 days after birth at the stage known as mini-puberty; the remainder undergo apoptosis. The lack of synchronization of these stages in the human fetal testis may serve to protect the number of spermatogonial stem cells (and thus future fertility) from various insults that may occur throughout fetal life.

In summary, in parallel with germ cell differentiation, a change in the expression of several proteins takes place. PLAP is positive mainly in young gonocytes and less in pre-spermatogonia. The largest number of positive cells is observed in the first trimester of gestation, decreasing as gestation progresses (Fig. 4.16). At birth PLAP is positive only in isolated cells (less than 1 per tubule) and located in the center of the tubule. C-kit is expressed in the second and third trimesters of gestation in a similar proportion in gonocytes and pre-spermatogonia. OCT3/4 is most expressed in the first trimester and the first half of the second trimester (4–6 cells per tubule), mainly in gonocytes. Its expression decreases to three cells per tubule in the second half of the second trimester. At the end of gestation, positivity is only observed in isolated gonocytes in the center of the tubule. TSPY is positive throughout gestation exclusively in spermatogonia. Ki67 is predominantly expressed in gonocytes up to the 24th week. From that week onward, the number of positive gonocytes and pre-spermatogonia is similar. The number of positive cells decreases as gestation progresses. At birth positivity persists only in isolated gonocytes in the center of the tubule, and pre-spermatogonia seem to have entered a quiescent period [97, 99] (Table 4.1).

GCNiS Cell Features in Childhood (Pre-GCNiS)

In childhood, seminiferous tubules with GCNiS have no lumen, and their size is similar to that of normal tubules. Gonocyte-like cells are located both in the center of the tubule and above the basement membrane. These cells show similar nuclear and cytoplasmic features to GCNiS cells in adults [100]. They occasionally show mitosis (Fig. 4.17). Like GCNiS cells, these pre-GCNiS cells are strongly positive for OCT3/4, TSPY, and PLAP and focally for SCF [101]. OCT3/4 suppresses apoptosis of gonocytes, TSPY enhances the proliferation of these cells, and SCF is a survival and growth factor for these cells [102].

Table 4.1 Evolution through fetal life of the immunoexpression of different markers in germ cells

Marker	Germ cell type	First trimester	Second trimester	Third trimester	First year of life
PLAP	Gonocyte	+++	++	+	+
	Pre-spermatogonia	++	+	–	–
c-KIT	Gonocyte	+++	+++	++	+
	Pre-spermatogonia	+++	+++	++	+
OCT 3/4	Gonocyte	+++	+++	+	+
	Pre-spermatogonia	–	–	–	–
TSPY	Gonocyte	–	–	–	–
	Pre-spermatogonia	++	++	++	++
Ki67	Gonocyte	+++	+++	+	+
	Pre-spermatogonia	++	++	+	–

+ Less than one positive cell per tubule (isolated), ++ 1–3 positive cells per tubule, +++ 4–6 positive cells per tubule

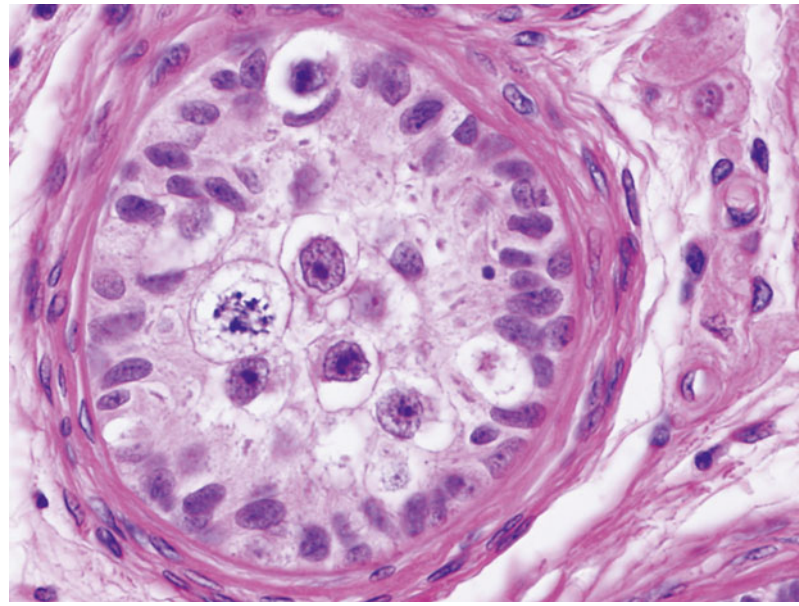

Fig. 4.17 Cross section of seminiferous tubule of a 9-year-old patient showing Sertoli cells in a pseudostratified arrangement and several large cells in the center, one of them in mitosis

GCNiS cells in children have DNA contents in a hypertriploid or hypertetraploid range [103, 104]. Seminiferous tubules with germ cells are arranged in small groups preferably beneath the tunica albuginea. Gonocytes in the center of the tubule retain an embryonic phenotype that persists beyond fetal development and childhood (OCT3/4 positive). These arrested gonocytes fail to differentiate due to impaired functioning of Sertoli cells in fetal development that prevents the movement of gonocytes from the center of the tubule to the periphery to get in contact with the basement membrane [105, 106]. This pattern is maintained until puberty, when the increase in hormone production produces changes in the spermatogonial niche to induce meiosis and haploid spermatozoa. At the same time that these changes take place, GCNiS cells start to proliferate. The result is a progressive transformation of a GCNiS cell phenotype from infantile to adult.

Delayed Maturation of Gonocytes Versus Infantile GCNiS

The finding of gonocyte-like cells in an infantile testis after the third month of postnatal life, when

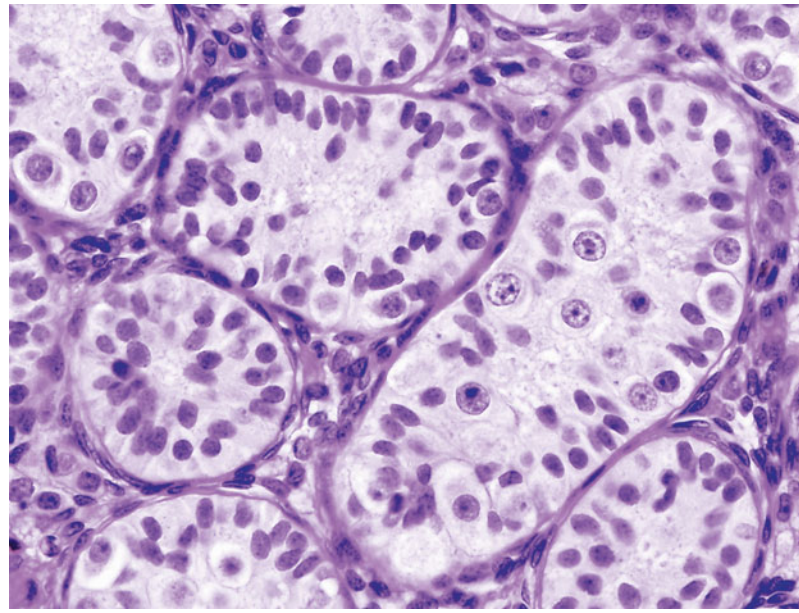

Fig. 4.18 Infantile testis of a patient with 46XY disorder of sex development with Müllerian remnants showing seminiferous tubules with intratubular gonocytes both above the basement membrane and at the center of the tubule

mini-puberty finishes, raises the possibility of a delay in the maturation of gonocytes instead of an infantile GCNiS. This differential diagnosis is of great prognostic importance since the malignant potential of delayed maturation of gonocytes is practically nonexistent, while infantile GCNiS has a high risk of malignant evolution [101, 107, 108]. The problem becomes more difficult when these cells appear in populations with a high risk of GCTs, such as individuals with gonadal dysgenesis, Down syndrome, and undervirilization syndromes [109]. The differential diagnosis should be supported by the age of the patient together with cytologic, histologic, immunohistochemical, and ploidy marker data. Gonocytes with delayed maturation are uniformly dispersed in the entire testis and more or less randomly placed in the seminiferous tubules instead of in groups of seminiferous tubules (Fig. 4.18). These cells have cytologic features of gonocytes located both in the center of the tubule and above the basement membrane. In spite of their similar morphology to pre-GCNiS cells, the immunophenotype is different. OCT3/4 is expressed only in germ cells with delayed maturation that are in the center of the tubule and occasionally in the cells above the basement membrane. They are also PLAP positive (Fig. 4.19). The cells in the periphery of the tubule are TSPY positive as well. SCF is negative in delayed maturation. Inconsistent OCT3/4 expression in TSPY-positive cells in the absence of SCF expression indicates a delay in maturation.

Atypical Germ Cells that May Simulate GCNiS

Two types of germ cells can be misinterpreted as GCNiS cells because of their morphology, especially if the specimen has not been properly fixed. These cell types are hypertrophic and multinucleated spermatogonia. Both can be seen in the testicular parenchyma peripheral to GCTs in childhood.

Hypertrophic spermatogonia characteristically have large hyperchromatic nuclei without nucleoli and clear, abundant cytoplasm. They are polyploid cells that were probably arrested in the S phase of the cell cycle [110]. They are observed in cryptorchid testes and exceptionally in normal testes. Hyperchromatic spermatogonia are numerous in seminiferous tubules surrounding teratomas and have been observed around YSTs. These cells are negative for OCT3/4 and PLAP and positive for PCNA and p53, which is a sign of their non-tumor-proliferative nature [9, 111].

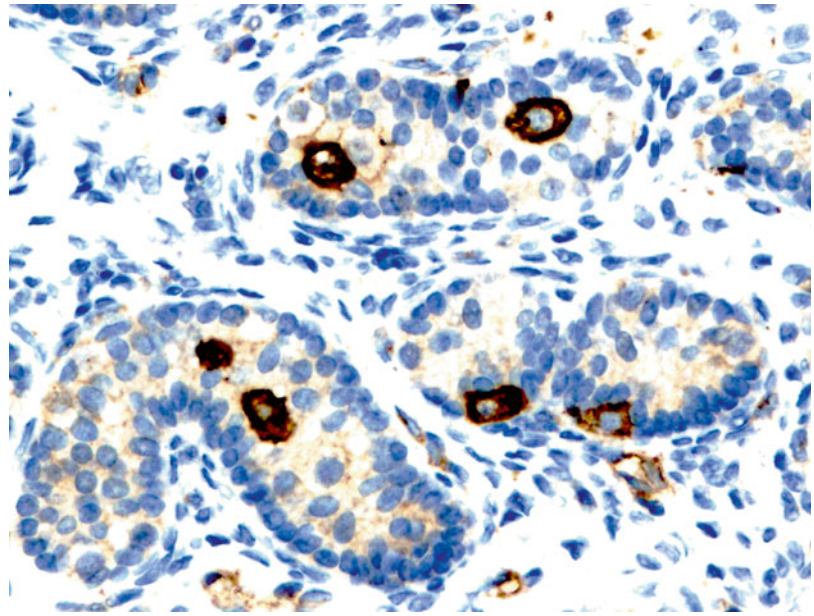

Fig. 4.19 PLAP-positive cells inside a seminiferous tubule

Multinucleated spermatogonia commonly show three or four nuclei, but the number can be higher. The nuclei can show features of spermatogonia type A dark, A pale, or a mixture of both. Multinucleated spermatogonia have been observed in cryptorchid testis with or without GCNiS. They have not been reported in normal testes. Multinucleated spermatogonia are produced by amitotic cell division [112].

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