Essentials of Diagnostic Gynecological Pathology
Series Editors: Naveena Singh · W. Glenn McCluggage

Nafisa Wilkinson *Editor*

Pathology of the Ovary, Fallopian Tube and Peritoneum

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This series is dedicated to the respected memory of Professor Harold Fox (1931–2012), one of the foremost gynecological pathologists of all time, in recognition of the ageless legacy of his teaching and written word which will continue to inspire many generations of gynecological pathologists.

Preface to the Series

This is a most exciting time to be submitting the second volume in the *Essentials of Diagnostic Gynecological Pathology* series for publication. Over the last decade there have been dramatic changes in the way we think about ovarian cancer. Ovarian epithelial cancer is no longer considered to be a single disease, but has been shown to comprise five major disease subtypes, distinct in their pathogenesis, pathology and clinical behaviour. It has become apparent that the most common type of 'ovarian' cancer, high grade serous carcinoma, probably originates in the fimbrial end of the fallopian tube in the majority of cases. A new FIGO staging system for ovarian cancer has recently been published, combining the staging of ovarian, tubal and primary peritoneal cancers, in recognition that distinction between these has no clinical or therapeutic relevance. The 2014 WHO 'blue book' on ovarian tumors is eagerly awaited, formalising all of these changes.

Gynecological pathology forms a large part of the diagnostic pathology workload of most histopathology laboratories. Research findings result in changes in diagnostic criteria and staging systems as well as descriptions of new ancillary techniques and diagnostic entities. Pathologists need to keep up to date with these changes, in view of the ever increasing pressure from clinicians, the public and the health service provider for accurate and timely diagnosis. The female genital tract is a complex system with several different organs, and in-depth knowledge of all areas is not necessarily the requirement of all pathologists.

The British Association of Gynecological Pathologists (BAGP) supports the development and maintenance of the highest standards of practice in diagnostic gynecological pathology. This is achieved through various educational activities including courses, workshops, meetings, cases of interest (www.thebagp.org) and quick dissemination of information on new developments via email to its members. The sponsorship of this textbook series is a part of this educational campaign. The series is intended for consultant and trainee pathologists, as well as clinicians and researchers, who may have a requirement for in-depth and up-to-date information on one area of Gynecological pathology rather than all areas. Those with an interest in all areas will benefit from division of topics into smaller volumes concentrating on different parts of the female genital tract. These multi-author textbooks have the advantages of being more affordable and amenable to timely and responsive updating alongside major practice changes in the field of Gynecological pathology.

We hope that this volume will be a useful addition to the existing literature on the subject. This textbook acknowledges the new histotype approach to ovarian cancer diagnosis and will be one of the first texts to incorporate the 2013 FIGO staging system, together with providing practical guidance on the assignment of primary site in cases of high grade serous carcinoma where multiple sites are involved. It is our intention that, like its successful predecessor, *Pathology of the Vulva and Vagina*, this volume will present the reader with the essentials of diagnostic ovarian, tubal and peritoneal pathology in a compact but comprehensive format.

Belfast, UK — W. Glenn McCluggage, FRCPath
London, UK — Naveena Singh, FRCPath

Preface to This Volume

The pathology of the ovary, Fallopian tube, and peritoneum is a vast area, and it is difficult to cover this entire subject comprehensively, but we have attempted to try and provide the kind of information that a diagnostic histopathologist would need at their fingertips when involved with making a diagnosis of an ovarian neoplasm. With this in mind, we have covered diagnostic aspects and the handling of biopsies as well as per operative frozen sections with a consideration of pitfalls. A great deal of information can be gleaned from the examination of the resected ovarian tumor specimen and the examination of the gross specimen cannot be overemphasized. This aspect is explored in a chapter on "cut up." The individual types of ovarian neoplasms are discussed in detail with a generous use of illustrations.
There have been many recent and exciting developments in the pathology of the ovary, Fallopian tube, and peritoneum, which makes this volume timely. The amendments to the FIGO staging of the ovary and Fallopian tube have been incorporated, as have the new ideas regarding the origin of ovarian carcinoma in particular high-grade serous carcinoma.

In the United Kingdom, carcinoma of the ovary and Fallopian tube is managed within cancer centers supported by a robust multidisciplinary team (MDT). MDT meetings are an extremely valuable source of clinical information, including a way of obtaining the past medical history, particularly if the patient has had a previous neoplasm. It is by working as a team with the gynecological oncologists, medical oncologists, clinical oncologists, and of course radiologists that we can endeavor to offer a high-quality diagnostic service to patients with ovarian cancer. Ultimately, a better understanding of the disease and its behavior will lead to targeted therapy and improved survival of patients with this disease. It is likely that the pathologist will play a role in determining sensitivities of ovarian tumors by immunohistochemistry when targeted therapy becomes normal practice.

We have, I hope, in this volume managed to capture some of the major changes in ovarian tumor pathology and incorporate them into what we would like to become a useful benchbook for the diagnostic histopathologist with some useful information regarding the management of the patient.

Leeds, UK — Nafisa Wilkinson, MA, MBBChir, FRCPath

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I would like to thank Naveena Singh and Glenn McCluggage for inviting me to take on the task of editing this volume and all the authors who have so generously given their time to research and write these chapters. Joanna Bolesworth and Joni Fraser have been great to work with. I would like to thank them in particular for their persistence with weekly teleconferences as the project was coming to conclusion. Steve Toms and Michael Todd have given a lot of support with the illustrations for which I am most grateful. Finally I would like to thank my husband Mark and son Christopher who tolerated me as I worked in the evenings.

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Anatomy, Development, Histology, and Normal Function of the Ovary

1

Nicolas M. Orsi, N. Ellissa Baskind, and Michele Cummings

Abstract

The ovary is an unique organ which fulfils a plethora of physiological functions ranging from its well-recognized roles in steroid hormone biosynthesis, oocyte production and the support of early pregnancy through to its rather less well-known involvement in the regulation of growth, behaviour and immune function in response to an array of endogenous and environmental cues. While multiple cytokine-mediated interactions between the granulosa/theca cell compartments and the oocyte of developing follicles are central to the coordination of ovarian cyclicity, its systemic effects are instead attributable to exquisitely well-honed interactions between ovarian follicles, the hypothalamus and pituitary implicating an array of peptide and steroid hormones. During the follicular phase, primordial follicles are called upon to initiate a process of follicular development which culminates in ovulation. However, the release of an oocyte belies the fact that the postovulatory follicle has yet another role to play in the luteal phase. Therein, it adopts a new keystone position in supporting the establishment and maintenance of pregnancy through its production of progesterone. Once the ovary has exhausted its reserve of follicles throughout reproductive life, many of its functions are lost as women enter the menopause. This Chapter aims to explore the ovary's developmental origins and functional roles to highlight the complexity of this remarkable product of reproductive evolution.

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Introduction

The ovaries have long been recognized for their endocrine and reproductive functions, embodied by their role in steroid biosynthesis and oocyte production, respectively. Their functional subunits, the oocyte-bearing follicles, are composed of multiple somatic cell types which

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communicate through extensive paracrine cytokine/growth factor-mediated networks to orchestrate oocyte development and cyclical steroid hormone production in response to both host-specific and environmental cues. However, the function of the ovaries extends beyond the regulation of reproductive cyclicity and oocyte production: their central role in female endocrine status also places them center stage for functions as diverse as regulating body growth, behavior, appetite, immune function, and the establishment of pregnancy. This range of functions is maintained by the dynamic role of the ovary in physiological homeostasis which, in turn, is governed by the structural arrangements and function of its follicles' theca and granulosa cell compartments.

Ovarian Anatomy

Human ovaries are paired, flattened, and ovoid bodies which lie adjacent to the lateral pelvic wall just inferior to the pelvic inlet [1, 2]. These whitish structures are approximately 3–5 cm long, 2 cm wide, and weigh 2–3.5 g and, based on age and ovarian cycle stage, can have either a smooth or puckered/nodular, uneven surface. The ovaries feature both lateral and medial surfaces, superior (tubal) and inferior (uterine) poles, and anterior (mesovarian) and posterior (free) borders [3]. In nulliparous women, each is suspended in the ovarian fossa (a shallow depression on the lateral pelvic wall) by its own mesentery, the mesovarium, which runs bilaterally from the posterior aspect of the broad ligament of the uterus (posteriorly and inferiorly to the uterine tubes to the lateral pelvic wall at the bifurcation point of the common iliac artery) [1–3]. In the erect posture, the long axes of the ovaries are vertical. This anatomical location is variable as the ovaries are frequently displaced outside the ovarian fossa during the first pregnancy, a change believed to be permanent [3].

The ovaries are unusual in being the only pelvic structure that is extraperitoneal, as highlighted by the fact that their surface cuboidal/columnar germinal epithelium (of Waldeyer) is a modified peritoneal covering continuous with the surrounding mesothelium of visceral peritoneum [1, 3]. The transition between the peritoneum's squamous epithelium and that of the ovary is usually marked by a line around the anterior border of the gonad [3]. The germinal epithelium overlies a layer of connective tissue-based tunica albuginea which, in turn, covers the cortical ovarian stroma and its resident follicles at various stages of development (see later) (Fig. 1.1). Surrounding these is a matrix containing various cell types, including fibroblasts, smooth muscle cells, and vascular networks. By contrast, the medullary core is composed of irregular, dense connective tissue which contains the gonad's vascular, lymphatic, and nerve supply, accounting for its original name of zona vasculosa of Waldeyer [1, 3]. This stroma also forms the hilar tissue which provides the entry point for vessels and nerves as well as the ovary's attachment [3].

The tubal pole lies near the external iliac vein, and over it are draped the fimbriae fringing the ostium of the fallopian tube, whose role is to capture the oocyte at ovulation. This pole is also connected to the suspensory ligament, a fold of peritoneum directed upward over the iliac vessels which runs to the lateral wall of the pelvis [1–3]. By contrast, the narrower uterine pole faces the pelvic floor and connects to the lateral angle of the uterus via the ligament of the ovary proper, which lies within the broad ligament. The position of the ovary within the pelvis varies considerably – testimony to this is the fact that it is frequently found prolapsed into the pouch of Douglas even in women with an otherwise normal anatomy [1].

The suspensory ligament of the ovary contains the gonad's neurovascular and lymphatic supply [1, 2]. The principal arterial supply is via the ovarian artery which traverses the mesovarium to enter at the ovarian hilum. This vessel anastomoses extensively with the tubal branch of the uterine artery in the mesosalpinx, thereby also providing branches to the uterine tube. Venous drainage emerges from the hilum in the form of a pampiniform plexus akin to that of the testis. The ovarian vein is formed from this plexus within the broad ligament and leaves the pelvis following the course of its homonymous artery [3]. Although the right ovarian vein drains directly into the inferior vena cava, its left counterpart drains into it via the intermediary of the left renal vein. Lymphatic vessels meet their counterparts from the uterine tube and fundus to form the lumbar lymph plexus.

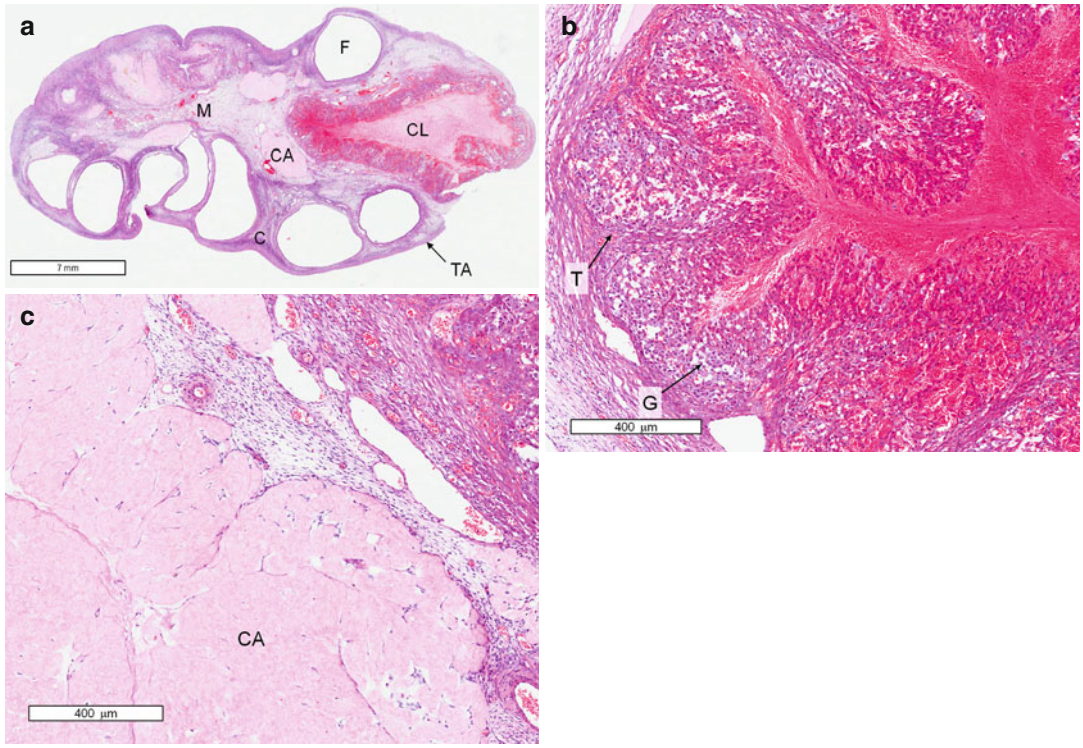

Fig. 1.1 (**a**) Normal ovary from a non-pregnant, pre-menopausal woman (H&E, ×20). The ovary is encapsulated by a layer of fibrous connective tissue, the tunica albuginea (*TA*). The body of the ovary (ovarian stroma) consists of specialized spindle-shaped cells and a smaller amount of ordinary connective tissue. The core of the ovarian stroma (the medulla; *M*) contains the ovary's main vessels, whereas the peripheral zone (the cortex; *C*) contains follicles (*F*) at various stages of development. Following ovulation, the ruptured follicle collapses to form the progesterone-secreting corpus luteum (*CL*). If fertilization or implantation do not occur, the CL degenerates to form the fibrous corpus albicans (*CA*). (**b**) Corpus luteum (×80). Granulosa (*G*) and theca (*T*) lutein layers form from the zona granulosa and the theca interna, respectively. Granulosa luteal cells are rich in lipid droplets, which are used as substrates for steroidogenesis (progesterone biosynthesis principally). Theca luteal cells form a thin zone around the granulosa layer, into which they project. Theca luteal cells are smaller, stain more densely, with less vacuolated cytoplasm and are responsible for estrogen biosynthesis. The CL is surrounded by a connective tissue zone (C), which arises from the theca externa. (**c**) Corpus albicans (×80). The corpus albicans is a steroidogenically inactive fibrous tissue mass resulting from the involution of the CL in the absence of pregnancy, whose secreting cells have been scavenged by macrophages and whose supporting vascular tissue has regressed and merged with the surrounding ovarian stroma. Corpora albicantes are abundant in the human ovary, and their number increases with age

The ovary also has a sympathetic innervation which runs alongside its vascular supply and connects to the pelvic plexus. A similar course is followed by parasympathetic fibers which are derived from a pelvic splanchnic supply [2].

Embryological Origins of the Ovary

The development of the ovaries starts early during postimplantation development with the appearance of primordial germ cells (PGCs) during gastrulation. These are first identifiable in the posterior rim of the embryonic disk and, subsequently, in the intermediate mesoderm, visceral mesoderm, yolk sac, and allantois [4]. During the 5th week of development, PGCs start their migration from the yolk sac using amoeboid movement and travel caudally via the dorsal mesentery to populate the mesenchymal urogenital ridge of the posterior body wall near the 10th thoracic level, medial and ventral to the developing mesonephric kidney. Their arrival stimulates the surrounding coelomic epithelium and mesoderm to proliferate

and thicken to form the genital ridges [4, 5]. These are the site of origin of the primary sex cords (composed of germinal epithelium growing into the underlying mesoderm) which develop into the weakly formed rete ovarii, which is succeeded by the secondary sex cords awaiting PGC colonization. During the 6th week of development, coelomic epithelial cells generate aggregates of somatic supporting cells which progressively envelop the PGCs. In parallel, the paired Müllerian (paramesonephric) ducts form laterally to their mesonephric counterparts by the craniocaudal invagination of a ribbon of thickened coelomic epithelium to reach the posterior wall of the urogenital sinus caudally. These are completed by the end of the 6th week, at which stage both male and female presumptive gonads remain indistinguishable from each other. Initially, both the genital ridge and mesonephros share a common mesentery, but as development progresses, the former largely severs its contact, remaining connected by a peritoneal fold, the mesovarium [4].

Sexual differentiation becomes evident from the 7th week when the absence of the Y chromosome's SRY gene expression enables the basic female developmental pathway to culminate in ovarian development [6]. While the PGCs will ultimately give rise to oogonia, somatic complement cells delaminating from the coelomic epithelium give rise to follicle pre-granulosa and theca cells (TCs) (these two cell types are counterparts of the male Sertoli and Leydig cells, respectively). The allied deficient anti-Müllerian hormone and testosterone production allows the Müllerian ducts to persist (ultimately giving rise to the fallopian tubes, uterus, and upper vagina) while the male-pattern pathway mesonephric ducts degenerate [4]. Throughout the 4th month, the histoarchitecture of the gonadal cords is progressively disrupted. The mitotic division of germ cell nuclei with incomplete cytokinesis forms multinucleated syncytia known as germ cell nests. Through a combination of germ cell apoptosis and somatic cell migration, germ cell nuclei are packaged with their pre-granulosa complement to form the initial primordial follicle pool [5]. The proliferation of these oogonia is tightly regulated by transforming growth factor (TGF)-β family mitogens, including activin, bone morphogenic proteins (BMPs), and TGF-β_1, before these eventually enter their first meiotic division to give rise to primary oocytes [7, 8]. This triggers adjacent gonadal cord cell-derived somatic support cells to differentiate into granulosa cells (GCs): as these form flattened monolayers surrounding individual oocytes, they form the first primordial follicles which, by the 5th month, are clearly visible in the cortical/perimedullary zone [8]. By contrast, the ovarian medulla becomes the focus of neoangiogenic, neural, and supportive connective tissue development [4].

The development of oocytes that have completed the prophase of meiosis I (together with their follicle complement) then arrests until resumption is triggered by pubertal cyclical changes in pituitary gonadotropins. These primordial follicles constitute the entire female ovarian reserve, although recent findings regarding the isolation of ovarian oogonial stem cells from women of reproductive age undermine this long-established notion [9]. Estimates of primordial follicle numbers vary, but attrition attributable to massive germ cell loss reduces oocyte numbers from circa 6 million in the fetal human ovary to 300,000–2 million at birth [5, 10]. Of these, only around 40,000 will remain by the onset of puberty, of which 300–450 will mature throughout reproductive life, with the majority of later losses occurring through atresia (see later) [11, 12].

It is noteworthy that ovarian development starts in the posterior abdominal wall such that the ovaries have to later descend into the pelvis in the late 2nd to early 3rd month [4, 13]. The ovaries are drawn down by their respective gubernacula, bands of fibrous tissue derived from gonad-bound undifferentiated mesenchyme. Each of these runs from the inferior border of its developing ovary to the labioscrotal swellings [2, 13]. Unlike the descent of the testes, this process does not involve gubernacular shortening in females, such that the gubernacula develop in line with the rest of the body. As the gubernacula contact the uterine fundus at its junction with the uterine tube (as the developing Müllerian ducts) in the 7th week, however, this arrests the ovaries' progress which remain trapped in its peritoneal folds, while cranially, in the absence of androgens, the persistence of the cranial suspensory ligaments

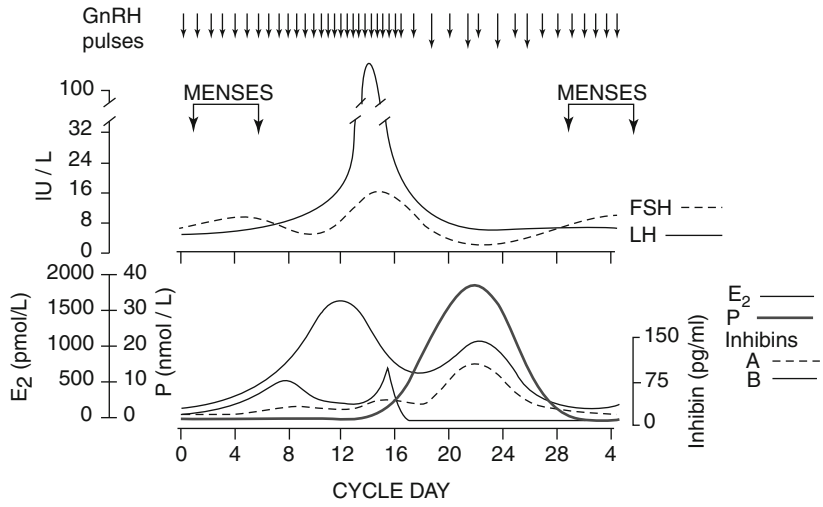

Fig. 1.2 The dynamics of GnRH (*upper panel*), gonadotropin (*middle panel*), and hormone (*lower panel*) profiles throughout the human menstrual cycle. *Key: E2* estrogen, *P* progesterone. (Reprinted from Marshall and Eagleson [325], with permission from Elsevier)

maintains the ovaries' anchorage to the abdominal wall [4]. The gubernacular remnants between the ovaries and the uterus ultimately develop into the ovarian ligaments proper bilaterally. Analogously, the remnants between the uterus and the fascia of the labia majora form the round ligaments of the uterus. Each of the latter is accompanied anteriorly within the recently formed inguinal canal by a peritoneal pouch (the canal of Nuck), which later obliterates and completes the developmental sequence [2, 4].

Physiological Function of the Ovary

The ovary has a dual function in the endocrine regulation of reproductive function and the production of gametes, both of which are interdependent. The *modus operandi* of the ovary is thus best understood in terms of cyclicity and by appreciating the details of follicular development.

Endocrine Function

Hypothalamic-Pituitary-Ovarian Control of the Ovarian Cycle

Women's reproductive lives are, without the intervention of hormonal contraception, characterized by a succession of menstrual cycles interspersed with pregnancy and lactation. The timing of each menstrual cycle (i.e., the interval between successive menses) is governed by the interval between successive ovulations (each of which occurs approximately 14 days prior to the onset of menses), the luteal phase being the most constant (Fig. 1.2). In humans, the ovarian cycle lasts 24–32 days and is traditionally divided into two phases characterized by follicular growth (the follicular phase) and that following ovulation (the luteal phase). The control of both peptide and steroid hormones which regulate these functions depends on a complex and dynamic feedback system involving the hypothalamus with its production of gonadotropin-releasing hormone (GnRH), the anterior pituitary with its secretion of glycoprotein gonadotropins (follicle stimulating hormone, FSH; luteinizing hormone, LH), and the ovary itself with its production of steroid (e.g., androgens, estrogen, and progesterone) and peptide hormones (e.g., inhibin)[1]. An assemblage of hypothalamic neurons makes up the GnRH pulse generator, which collectively coordinate the pulsatile secretion of GnRH into the

[1] Note: GC-derived inhibins sensitize TCs to LH, enhancing androgen production/estrogen synthesis. Other protein systems are involved: activin orchestrates FSH/LH receptor levels, estrogen synthesis, and oocyte maturation; follistatin inhibits activin-induced responses – the discussion of the finer points of their actions falls out with the scope of this more generic chapter and the reader is referred to references [14, 15] for a more detailed account.

hypophyseal portal system to reach its target pituitary gonadotropes. In response to stimulation, these produce both FSH and LH, although the frequency of GnRH pulsatility determines which is preferentially produced: slower pulses favor FSH while rapid pulses favor LH, accounting for their differential predominance in the early and late follicular phase, respectively [16–18]. Gonadotropins act on ovarian follicles to promote estrogen (FSH), progesterone, and androgen (LH) biosynthesis, which can then feed back to the pituitary to regulate both GnRH pulsatility and gonadotropin production. Progesterone slows GnRH pulse frequency and therefore LH secretion, while estradiol plays a permissive role in this regard, putatively through upregulation of hypothalamic progesterone receptors [19–21]. In the absence of steroid feedback, such as after the menopause or in premature ovarian failure, the GnRH pulse generator frequency returns to its baseline intrinsic firing frequency of circa one pulse per hour [22, 23].

The GnRH pulse generator is functional during intrauterine life but becomes relatively quiescent within 6–9 months of birth and maintains this hiatus until the prepubertal period. Childhood thus features a slow GnRH pulsatility which is reflected by low gonadotropin levels with a preponderance of FSH over LH [24, 25]. As this period draws to a close, nocturnal/sleep-related LH secretion becomes more prominent (i.e., as increased pulse amplitude/frequency), followed by an early diurnal production of estrogen and testosterone [26–29]. This circadian pattern is progressively lost by late puberty, putatively through physiological changes relieving inhibition by higher central nervous system pathways on GnRH pulse frequency and/or a gradual, androgen-driven loss of sensitivity to estrogenic feedback inhibition of LH secretion [30, 31].

The Follicular Phase

The follicular phase is dominated by growing follicle-derived estrogen and lasts approximately 10–14 days. Synthesis and secretion of gonadotropins from the anterior pituitary is regulated by pulsatile (~1/h) hypothalamic GnRH release. GnRH is self-priming such that the secretory response to a second pulse of GnRH is larger than the first [32]. In turn, FSH and LH secretion is modulated by both the amplitude and frequency of GnRH pulses. Through negative feedback, estradiol normally suppresses FSH and LH secretion, both directly by acting on the hypothalamus and indirectly by altering the number of pituitary GnRH receptors [33].

At the time of menses, circulatory levels of estrogen and progesterone are relatively low such that negative feedback is relaxed. This causes a rise in FSH and LH levels which support follicular development and growth which, in turn, results in an increased production of estrogens and inhibin. If estradiol levels increase markedly (e.g., 200–400 % more than in the early follicular phase) and remain high for ~48 hours, then FSH and LH secretion is enhanced rather than being suppressed (i.e., generating a positive feedback loop) [34]. Thus, in the latter part of the follicular phase, the surge in estradiol levels initiates positive feedback on the hypothalamus and pituitary and thus results in a rapid increase in gonadotropin levels. The theca interna solely expresses LH receptors; in response to LH, these cells convert cholesterol to androgens [35]. These steroids diffuse to the GCs below where they play a dual role: while they are substrates for aromatase, they also increase the activity of this enzyme, thereby increasing estrogen production [36, 37]. Estrogen production occurs in response to FSH, whose receptors are expressed by this cell type. In addition, estrogen acts in a paracrine manner on GCs, stimulating both their proliferation and estrogen biosynthesis, thereby establishing a local positive feedback [38, 39].

A rise in both FSH and LH heralds the onset of the preovulatory phase which is matched by a transient rise in androgens and estrogens [40]. In particular, the LH surge induces terminal growth and maturation of both the dominant follicle, whose follicular fluid (FF) volume rapidly increases, and its oocyte, which resumes meiosis. The combination of estrogen and FSH promotes LH receptor expression on GCs which forfeit estrogen production in favor of the LH-stimulated progesterone synthesis which characterizes the luteal phase (below).

The Luteal Phase

The luteal phase which follows ovulation is characterized by a corpus luteum-derived progestogen dominance and lasts circa 12–15 days. Although significant quantities of ovarian estrogens and androgens are still produced, high progesterone levels enhance the negative feedback effects of estrogens on both the hypothalamus and pituitary such that FSH and LH production is dramatically reduced. Progesterone has a range of systemic effects such as immunomodulation and appetite/behavioral changes, although it is mostly recognized for its induction of endometrial gland secretion to favor blastocyst implantation in the estrogen-primed uterus [41].

At the end of the luteal phase, if the corpus luteum regresses in the absence of an embryo, the accompanying decline in estrogens and progesterone relieves feedback inhibition and enables FSH and LH levels to rise once again. In parallel, withdrawal of ovarian steroid support triggers the onset of menses and the endometrial lining is shed in preparation for renewed estrogen-dependent growth during the subsequent follicular phase [42].

Folliculogenesis and Ovarian Histology

Over the last two decades, the ovary has increasingly been appreciated to be a functional unit governed by the active and highly complex dialogue between oocytes, GCs, TCs, stromal cells, and infiltrating immune effector cells, both within and across follicles [43–45]. This hormone-sensitive cross talk whose mediators are still being identified is essential to support all stages of oogenesis, folliculogenesis, and ovulation [46–50].

Folliculogenesis occurs in the ovarian cortex and begins with the recruitment of a primordial follicle into the pool of growing follicles and ends with either ovulation or death by atresia; the entire process can take up to a year in women. Folliculogenesis is divided into two phases. The first phase, termed the pre-antral or gonadotropin-independent phase, is characterized by the growth and differentiation of the oocyte and is principally controlled by locally produced growth factors through autocrine/paracrine mechanisms. This phase covers the transition of the primordial follicle to the primary pre-antral follicle. By contrast, although the second phase also features continued follicular growth, this occurs in response to pituitary gonadotropins (Fig. 1.3).

The Primordial Follicle

The oocyte in the primordial follicle measures 25 μm in diameter and is arrested in the dictyate stage of meiosis. It is surrounded by simple squamous epithelium composed of a single layer of flattened GCs and a basal lamina which enables the oocyte and GCs to coexist within a microenvironment isolated from direct contact with other cells. Paracrine signaling and nutrition occur by diffusion since primordial follicles lack their own vascular supply and thus have limited access to endocrine support [51]. Follicular recruitment is marked by a change in GC morphology from squamous to cuboidal together with increased mitotic activity and oocyte growth. Follicular recruitment starts during fetal life and continues at a relatively constant rate during the first three decades of life, with 20 or so primordial follicles being recruited during each ovarian cycle. This process is accompanied by atresia of non-growing follicles which accelerates over time, resulting in an overall decrease in ovarian reserve and reduced fecundity by age 30 years and a marked decrease by age 35 years [52].

Various intrafollicular cytokines participate in regulating primordial follicle activation and transition to the primary follicle stage, including leukemia inhibitory factor, basic fibroblast growth factor (bFGF), stem cell factor, stromal derived factor-1, platelet-derived growth factor, and BMP-4 [53–57] (Fig. 1.4). This growth factor network is supported by surrounding stromal cell-derived mediators, such as keratinocyte growth factor, BMP-4 and BMP-7 [56, 58]. In addition, GC-derived anti-Müllerian hormone mediated dialogue also occurs in order to inhibit adjacent follicle activation [59–61].

The Primary Follicle

The primary follicle initially features a single layer of cuboidal GCs surrounding the oocyte which proliferate rapidly to give rise to the stratified

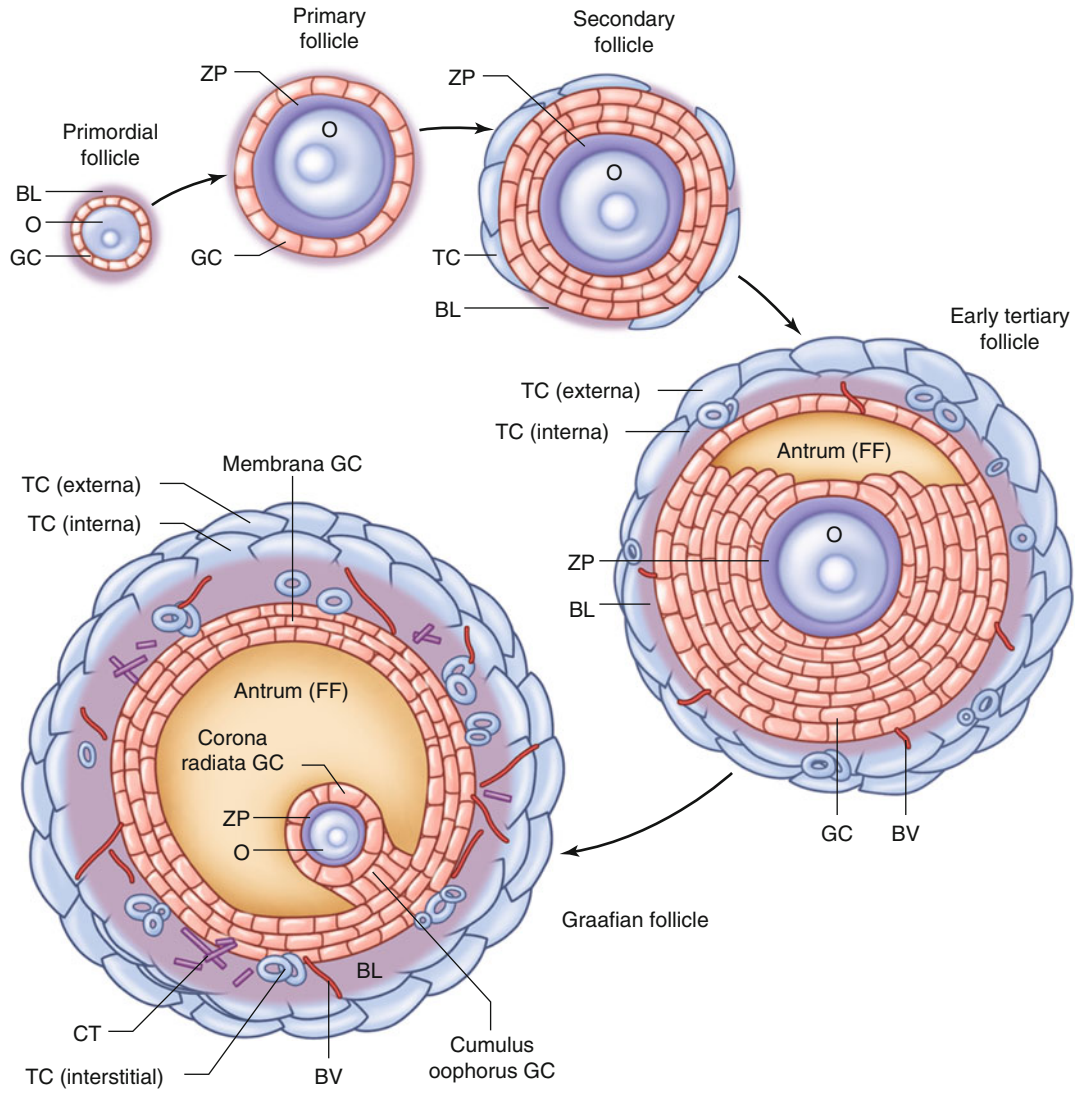

Fig. 1.3 Schematic representation of development of the primordial follicle to the pre-ovulatory Graafian follicle. *Key*: *BL* basement lamina, *O* oocyte, *GC* granulosa cell, *ZP* zona pellucida, *TC* theca cell, *FF* follicular fluid, *BV* blood vessel/capillary, *CT* connective tissue (loose)

zona granulosa (Fig. 1.5). This stage of development is characterized by an upregulation of FSH receptor expression and increased oocyte growth [62]. Following genomic reactivation, the oocyte increases in size considerably and acquires the zona pellucida, a glycoprotein shell from which the blastocyst will ultimately hatch prior to implantation [57]. The oocyte nevertheless retains functional contact with its surrounding GCs through the development of intimate intercellular connections [47, 63]. Cytoplasmic projections and microvilli grow from both the oocyte and the GCs and interdigitate to create a large surface area for diffusion [64]. Gap junctions established in these areas of cell-cell contact facilitate the diffusion of ions, metabolites, and signaling molecules [65]. The stroma surrounding late primary follicles begins to organize into the theca folliculi, which is separated from the zona granulosa by a basement membrane. Subsequent development of primary follicles to fully grown secondary follicles results from an active autocrine/paracrine regulatory process dependent on oocyte-

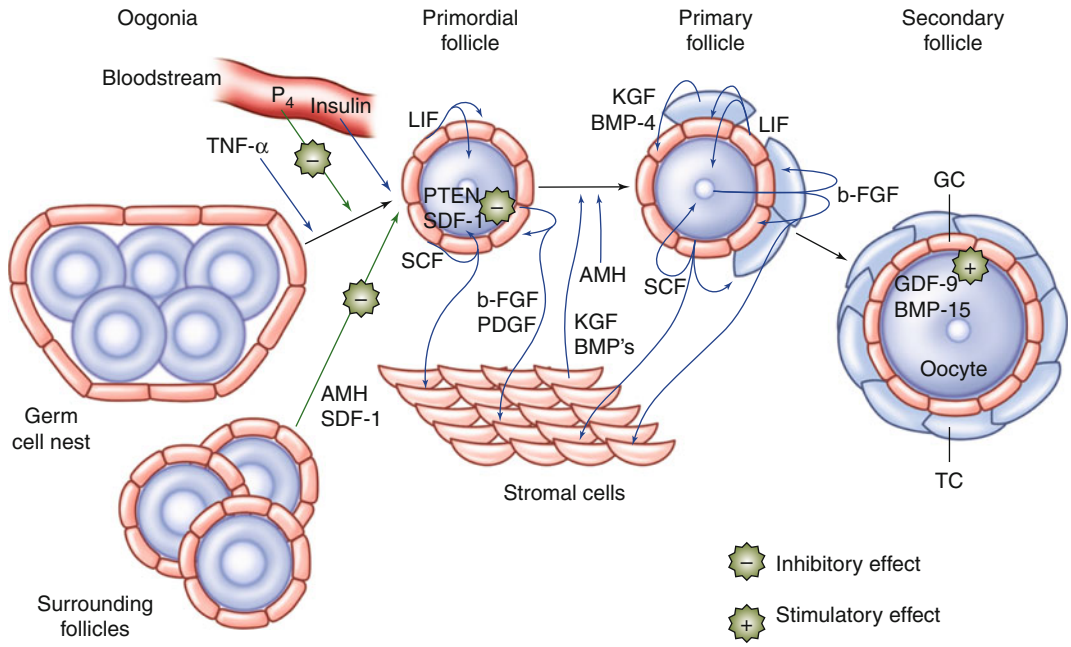

Fig. 1.4 Schematic representation of the predominant regulatory factors governing primordial follicle transition to the primary follicle in the ovary. The combined actions of primordial follicles themselves, surrounding stromal cells, follicles and endocrine factors are responsible for primordial follicle activation. The primordial follicle oocytes express PTEN and SDF-1 which inhibit their own activation, and secrete PDGF and b-FGF which stimulate pre-granulosa cells to generate KL (SCF). KL then promotes oocyte growth and follicle activation, as well as stromal cell recruitment. Follicle activation is further stimulated by KGF, BMP-4, and BMP-7 secreted by adjacent stromal cells. Neighboring follicles secrete AMH and SDF-1 which negatively regulate primordial follicle activation. Circulatory insulin contributes further to follicle activation. Once primary follicle status has been attained, the GCs continue to express KL, while the oocytes secrete GDF-9 and BMP-15, which promote GC proliferation, KL secretion and theca formation to support the formation of the secondary follicle (whose structure has been simplified for illustrative purposes). LIF, expressed by the GCs of the primordial and primary follicle interacts with KL to promote primordial follicle transition and subsequent development of the primary. Progesterone inhibits follicle assembly, while TNF-α promotes apoptosis which is key to follicle assembly. *Key*: *TNF-α* tumor necrosis factor, *P* progesterone, *SDF-1* stromal derived factor-1, *b-FGF* basic fibroblast growth factor, *KL* kit ligand (stem cell factor (SCF)), *LIF* leukemia inhibitory factor, *KGF* keratinocyte growth factor, *BMPs* bone morphogenic proteins, *AMH* anti-Müllerian hormone, *PDGF* platelet derived growth factor, *PTEN* phosphatase and tensin homologue, *GDF-9* growth differentiation factor-9, *GC* granulosa cell, *TC* theca cell

derived cytokines. Ovine and murine animal models suggest that growth differentiation factor (GDF)-9 and BMP-15 are crucial in this step; in the absence of these mediators, follicle growth and development arrest at the primary follicle stage [66].

The Secondary Follicle

Secondary follicles are usually situated deeper in the ovarian cortex. As the follicular developmental continuum progresses, active GC proliferation results in the formation of a stratified or pseudostratified columnar epithelium surrounding the oocyte which has by now almost reached its full size (120 μm). Within this, small fluid-filled vacuoles appear, gradually causing the oocyte and its surrounding GCs to become eccentrically positioned. These eventually coalesce by the tertiary follicle stage. The surrounding theca cell layer progressively differentiates into two primary layers: an inner theca interna comprising rounded interstitial cells and an outer theca externa that differentiates into spindle-shaped smooth muscle cells [67]. Theca development is accompanied by neoangiogenesis which promotes the development of a perifollicular vascular network within the layer which rapidly expands as the follicle increases in size (Fig. 1.6). In swine, this process has been noted to commence as the follicle

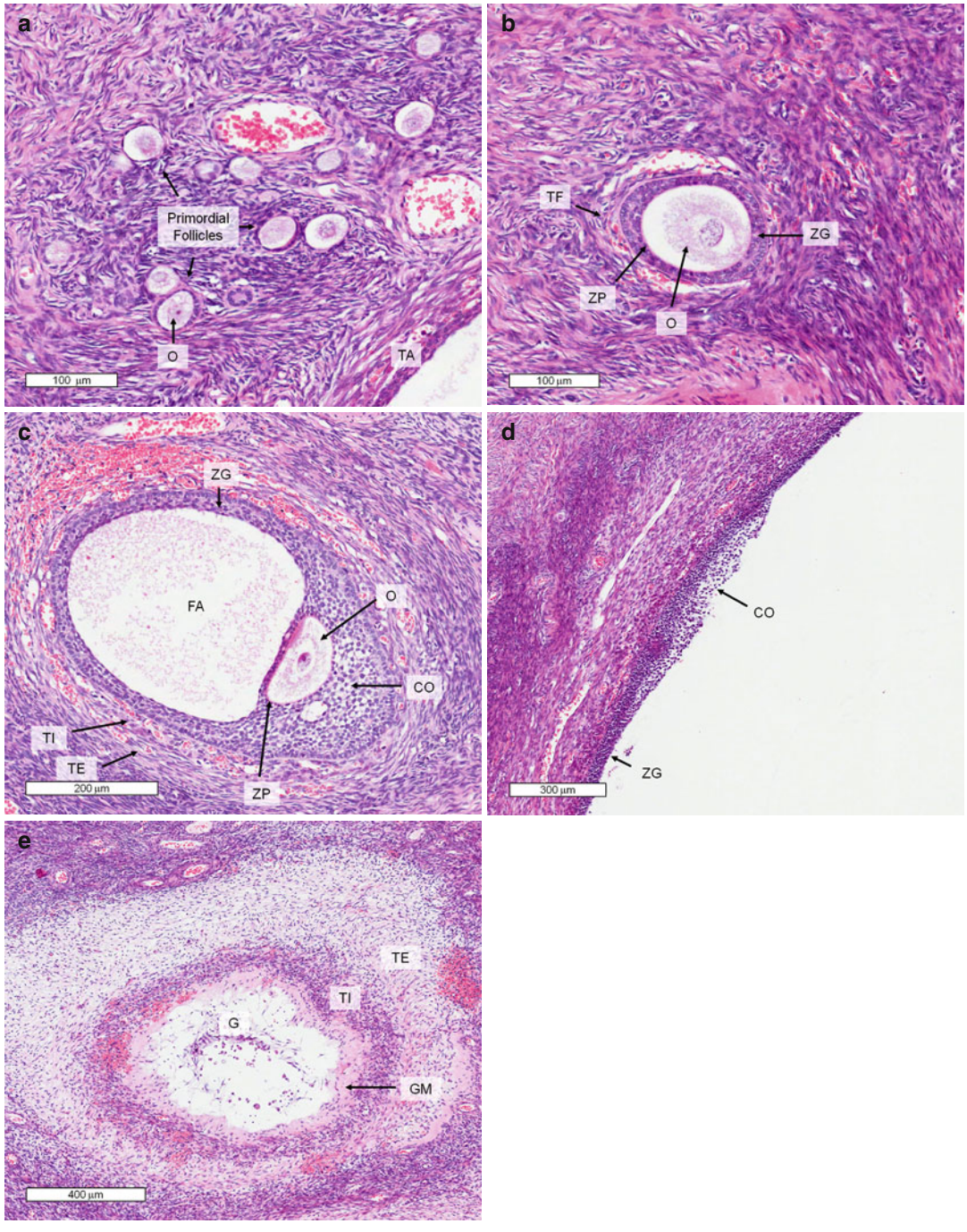
a
Primordial Follicles
O
TA
100 µm
b
TF
ZG
ZP
O
100 µm
c
ZG
FA
O
CO
TI
TE
ZP
200 µm
d
CO
ZG
300 µm
e
TE
TI
G
GM
400 µm

Fig. 1.5 (**a**) Primordial follicles in the ovarian cortex (×200), consisting of a single layer of flattened follicular cells surrounding an oocyte (O) (*TA* tunica albuginea). (**b**) Primary follicle (×200): the oocyte (*O*) has greatly enlarged and a glycoprotein layer, the zona pellucida (*ZP*), develops between the oocyte and the surrounding follicular cells, which have now become cuboidal and proliferated to form a layer several cells thick (the zona granulosa, *ZG*). The connective tissue surrounding the follicle has begun to organize into a structure known as the theca folliculi (*TF*). (**c**) Late secondary/early tertiary (antral) follicle (×150). The follicle has continued to enlarge and a fluid filled follicular antrum (*FA*) has almost entirely formed from the coalescence of individual vacuoles, such that the oocyte (*O*) is now positioned eccentrically. The granulosa layer surrounding the oocyte is known as the cumulus oophorus (*CO*), and its cells have become more loosely attached to the mural zona granulosa cells (*ZG*). The TF has differentiated to form two layers, the theca interna (*TI*), a site of steroidogenesis, and the theca externa (*TE*), a layer of smooth muscle cells. (**d**) Mature (Graafian) follicle (×80); oocyte missing in this section. The cumulus oophorus (*CO*) layer can be distinguished from the mural zona granulosa (*ZG*), as a thicker layer with more loosely attached cells. (**e**) Late atretic follicle. The oocyte and granulosa cells have degenerated, and the basement membrane separating the granulosa (G) and theca interna (TI) layers has thickened to form the glassy membrane (GM). This follicle likely reached the antral stage of development without becoming a mature (Graafian) follicle.

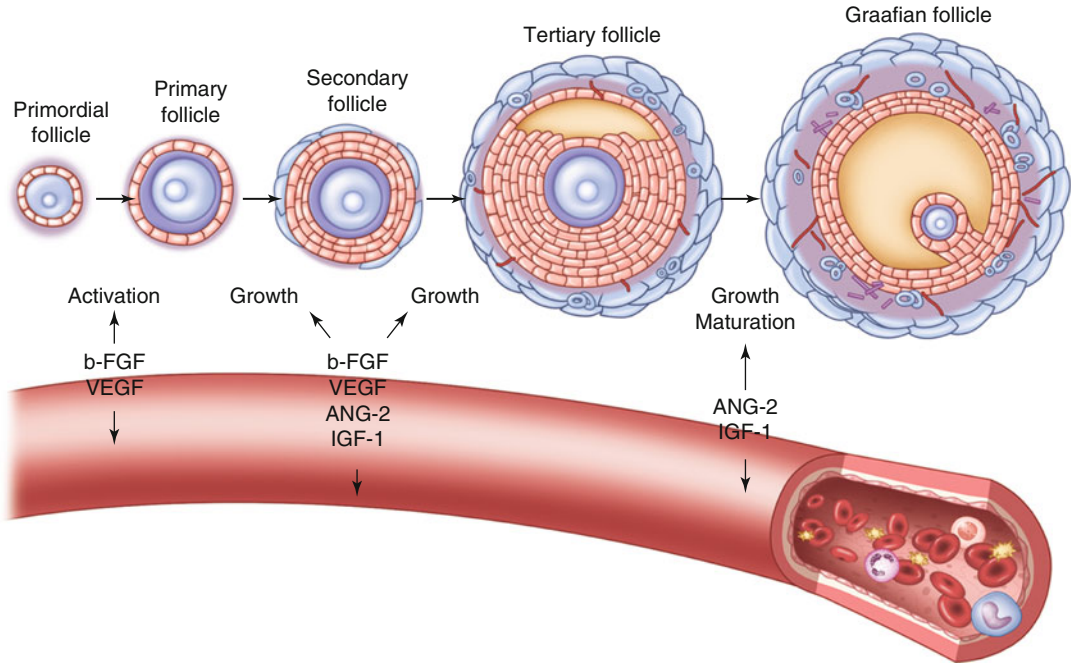

Fig. 1.6 Angiogenic growth factors acting at different stages in folliculogenesis. *Key*: *b-FGF* basic-fibroblast growth factor, *VEGF* vascular endothelial growth factor, *ANG-2* angiopoietin-2, *IGF-1* insulin like growth factor-1

diameter reaches 110 μm [68]. While angiogenesis is promoted by agents such as bFGF and vascular endothelial growth factor (VEGF) [69, 70, 71], overall vascular development is the product between the balance of these agents with inhibitory factors, including thrombospondin, angiostatin, endostatin, interleukin (IL)-8, 2-metoxiestradiol, hyaluronic acid, platelet factor-4, tumor necrosis factor (TNF)-α, interferon (IFN)-α, and IFN-γ [72–76]. In this respect, it is worth noting that angiogenesis is regulated independently across different follicles [77].

Although the oocyte completes its growth during pre-antral folliculogenesis, it does not normally resume meiosis. This meiotic arrest is believed to be under the inhibitory control of cyclic AMP (cAMP) and/or cyclic GMP [78, 79]. The development of a primordial follicle to a fully grown secondary follicle takes about 290 days.

The Antral Follicle

The second, antral (Graafian) or gonadotropin-dependent phase of folliculogenesis is characterized by the further follicular growth regulated by FSH, LH, and an extensive array of growth factors. A further 60 days is required for the Graafian follicle to develop. The most salient feature of these tertiary follicles is their evident cavity (antrum) which contains FF, a plasma transudate conditioned by secretory products from the oocyte, GCs, and infiltrating immune effector cells [80]. Although FF protein concentration is generally lower than that in the circulation (since the follicular wall acts as a coarse molecular sieve excluding proteins >850 kDa), amino acid concentrations are higher [81, 82]. The levels of various energy substrates and metabolites also differ markedly. Indeed, the energy metabolism of follicles is largely glycolytic, putatively in order to avoid oxygen being exhausted in the outer follicle layers in favor of supporting oocyte metabolism which relies heavily on the oxidative phosphorylation of pyruvate [83, 84]. Together with the absence of a vascular supply in the zona granulosa, this explains the comparatively high lactate concentrations found in FF, which are also believed to inhibit meiotic resumption through a decrease in pH [85–87].

Antral follicles are heterogeneous in size, measuring 0.4–25 mm in diameter, as their overall size is largely determined by the size of the antrum which is, in turn, dictated by FF volume (0.02–7.0 ml) [88]. The relative abundance of antral follicles and their sizes vary as a function of age and menstrual cycle phase. Antral follicle counts can be determined by ultrasound and are used clinically in the early follicular phase as a marker of ovarian reserve [89]. In general terms, it is accepted that in natural cycles the size of the Graafian follicle is directly correlated with oocyte maturity [90]. Bordering the antrum are distinct GC subtypes which exhibit individualized responses to FSH stimulation: the membrana, periantral area, and cumulus oophorus. Their subtype appears to depend on the relative GC proximity to the oocyte and exposure to oocyte-derived GDF-9 and BMP-15 [44, 57, 88]. While GCs and TCs proliferate extensively in the dominant follicle concurrently with antrum growth, FF formation and somatic cell mitosis ceases in atretic follicles, thereby limiting their size [57].

By now the theca is fully differentiated. The theca externa smooth muscle cells are concentrically arranged and autonomically innervated; their precise role remains unclear although it has been suggested that they may play a role in subsequent ovulation. By contrast, the theca interna is dominated characteristically by large epithelioid cells whose cytoplasm is filled with lipid droplets, smooth endoplasmic reticulum, and mitochondria with tubular cristae – the hallmark appearance of steroid-producing cells [67]. This reflects their role, from the time of antrum formation, in the production of high concentrations of androgens (e.g., androstenedione in response to LH and insulin stimulation), estrogen, and, in the preovulatory stage, progesterone, in addition to the FSH inhibitor inhibin F at the time of ovulation. The steroid hormones are responsible for promoting endometrial proliferation and secretion in preparation for subsequent blastocyst implantation. The theca thus becomes richly vascularized, with two independent capillary networks forming in the theca interna and externa, which collectively form the thecal

vascular sheath [57, 91, 92]. The gradual increase in angiogenesis in the developing follicle peaks in the late pre-antral/antral stage, supported by continued endothelial cell proliferation [93]. The growth and development of the theca vascular network is understood to be regulated through the coordinated expression of pro- and anti-angiogenic factors and their receptors [77, 94]. Interestingly, GCs predominantly synthesize these agents despite angiogenesis being confined to the thecal layer [77]. As outlined, GCs are separated from the theca by a basement membrane such that the metabolic requirements of GCs and oocytes must be met by either diffusion or active transport. The increase in vascularization which accompanies follicular growth is believed to be driven by hypoxia (and the associated increase in transcriptional complex hypoxia inducible factor (HIF)-1α activity) and prevents GC and oocyte compromise [95, 96]. Moreover, VEGF secreted by TCs increases vascular permeability and contributes both to the accumulation of antral fluid in the growing follicle and the delivery of the lipid precursors required for steroid biosynthesis [97–100].

As oocytes near the end of their growth phase, they acquire competence to undergo two aspects of maturation: cytoplasmic and nuclear. The former encompasses the processes that prepare the germinal vesicle-stage oocyte for activation and preimplantation development, viz., the accumulation of mRNA, proteins, substrates, and nutrients which provide the oocyte with the capacity to complete nuclear maturation [101–103]. By contrast, the latter refers to the reversal of meiotic arrest and progress to metaphase II [104, 105]. Nuclear and cytoplasmic maturation are coordinated by the mixing of germinal vesicle contents with the cytoplasm during germinal vesicle (GV) breakdown as the oocyte is prepared for fertilization and activation [106]. While somatic cells in the follicle can provide developmental cues that regulate oocyte maturation and prevent parthenogenetic activation, data from animal models also suggest that oocytes can also regulate GC cell proliferation and progesterone production while enabling the cumulus oophorus to synthesize hyaluronic acid and undergo expansion in response to FSH stimulation [107–112].

The preovulatory LH surge allows resumption of meiotic division and the progression of the oocyte from the dictyate stage to metaphase of the second meiotic division, resulting in extrusion of the first polar body. This contains very little cytoplasm and remains within the confines of the zona pellucida alongside the secondary oocyte. This response to LH in particular appears to relieve the inhibition exerted on the oocyte until then by follicular oocyte maturation inhibitor [113]. The cumulus oophorus regresses before ovulation, leaving the oocyte surrounded by a layer several cells thick, termed the corona radiata. Prior to ovulation, the remaining attachment of the cumulus-oocyte complex connection to the now markedly thinned zona granulosa breaks down altogether. By this stage, the follicle bulges under the ovarian surface. The overlying surface epithelium cells become flattened and atrophic and the thin intervening stroma becomes degenerate and avascular.

Follicle Selection and Atresia

In cycling women, the end of the luteal phase marks the selection of the dominant follicle from a cohort of early antral follicles. This process is under the influence of the secondary rise in plasma FSH subsequent to decreased estradiol and inhibin A production by the corpus luteum [114]. As the follicular phase proceeds, the dominant follicle grows rapidly in contrast to the remainder of its cohort and, in most species, acquires a greater blood supply [57, 115]. It is still a matter of some debate whether the regression in thecal vasculature associated with follicular atresia identified in animal models occurs as prominently in humans, although it has been proposed that this possible discrepancy may reflect the longer time taken for atresia to occur in human follicles [116].

The vast majority (99.9 %) of follicles undergo atresia, a process thought to be initiated by GC apoptosis controlled by both intrinsic and extrinsic pathways [117]. This process can occur in developing follicles after the secondary phase [57]. In the early stages, both theca and granulosa compartments remain largely intact. However, some loosened mural granulosa cells are found in the

antrum, the cumulus oophorus may be disrupted, the oocyte starts to degenerate, and the basement membrane appears to be thicker and folded. In the late stages, atretic follicles are shrunken, the basement membrane has further thickened and folded, and stroma has broadly replaced the follicular cells. While extrinsic pathway cytokines such as members of the TNF family (TNF-α, Fas ligand and TNF-related apoptosis-inducing ligand) are recognized inducers of cell surface death receptors in atretic follicles, FSH appears to be key in inhibiting intrinsic cell death in the dominant follicle [118]. Further evidence from animal models implicates cytokines such as GDF-9, BMP-6, and BMP-15 in the latter process through the inhibition of GC apoptosis and the promotion of cumulus cell survival, respectively [117, 119].

Ovulation

The dominant follicle is identifiable by its larger size, and accompanying its enhanced growth and greater vascularity is an increased blood peak flow velocity relative to its cohort [120]. Key to ovulation is the proteolytic degradation of the basal lamina and the follicle's apical connective tissue, which disrupts the follicular wall and allows oocyte release in response to the LH surge [121, 122]. This extensive remodeling of the extracellular matrix is attributable to the concerted activity of an array of matrix metalloproteinases (MMPs) whose expression profiles are decreased in the GC compartment in favor of its TC counterpart [123–125]. In turn, the activity of MMPs is controlled by their tissue inhibitors (TIMPs), whose production also increases in response to LH, albeit predominantly in the GC compartment [126–128]. While both MMPs and TIMPs are upregulated, MMP proteolytic activity is concentrated at the follicular apex, thus creating a weak point in the follicular wall which facilitates ovulation [129]. The LH surge also promotes an increase in progesterone, various cytokines (e.g., macrophage chemotactic protein-1, IL-1, IL-8, TNF-α), and prostaglandins (e.g., $PGF_{2\alpha}$), lending support to the notion that ovulation has many of the features of an inflammatory process [130–137]. Various animal models have implicated the action of additional mediators in increasing MMP expression, including relaxin and prolactin [138–141]. Through their control of localized MMP activity, TIMPs may have subsequent roles, such as stimulating cellular proliferation and/or promoting neoangiogenesis (which has until then been controlled by FF-dependent antiangiogenic factors) and steroidogenesis as the follicle luteinizes [129, 142–144]. They are likely to be assisted in this role via the LH-induced immune effector cell infiltrate and alterations in the local cytokine microenvironment.

As the weakened mature follicle wall ruptures at the stigma, the cumulus-oocyte complex is released near the entrance to the fallopian tube where ciliary action at the fimbriae sweeps this into the ostium. The cumulus-oocyte complex progresses to the ampulla where the oocyte is fertilized, completes its meiotic division and extrudes the second polar body while the remaining cumulus cells maintain an active paracrine role [145, 146]. Hemorrhage usually occurs into the remains of the follicle, which becomes a transient corpus hemorrhagicum before completing its development into the highly vascularized, lipid/lutein pigment-laden corpus luteum.

The Corpus Luteum

The onset of luteinization occurs in response to the preovulatory LH surge, which induces the terminal differentiation of follicular cells into steroidogenic lutein cells, whose activity primes the endometrium for embryo implantation [147–149]. Luteinization features the breakdown of the follicular basal membrane, which enables endothelial cells, fibroblasts, immune effector cells, and TCs to penetrate the GC layer, although the degree of GC-TC mixing remains limited in primates [149]. Thus, two relatively distinct populations of steroidogenically active cell populations can be distinguished in the corpus luteum: large, hypertrophic, and more centrally sited granulosa-lutein cells which produce progesterone and estrogen, and smaller, more peripherally located theca-lutein cells which are largely responsible for androgen production [150, 151]. These changes are facilitated by extensive extracellular matrix remodeling via the action of an

array of serine proteases, MMPs, and TIMPs, whose expression levels are governed in response to the LH surge and prolactin levels [152–155]. In response to an array of angiogenic molecular cues (VEGF, endocrine gland-derived VEGF, bFGF, angiopoietins), thecal pericytes invade the developing luteal parenchyma and provide the starting point for the extensive endothelial development which will ultimately allow each luteal cell cluster to be in direct contact with several capillaries, giving the corpus luteum one of the highest rates of blood flow and metabolic activities in the body [156–162]. This arrangement is essential in ensuring an adequate delivery of nutrients, hormones, and lipoprotein-bound cholesterol as well as maximizing the efficiency of progesterone export [162, 163].

The LH surge has widespread effects on ovarian endocrine signaling which occurs within hours [164]. This induces FSH receptor silencing, a transient LH receptor decline/desensitization, a brief progesterone receptor (which prolongs luteal lifespan by maintaining luteal paracrine progesterone signaling) and cyclooxygenase-2 upregulation, an increase in the prolactin receptor (which acts to prevent the premature expression of 20α-hydroxysteroid dehydrogenase (HSD), the enzyme responsible for the catabolism of progesterone to its inactive 20α-dihydro metabolite) and alterations estrogen receptor isoforms [165–168]. The hormonal control of luteal function is relatively complex and involves various key hormones. Firstly, in addition to its extraovarian roles, progesterone stimulates its own production, decreases its own catabolism, and exerts antiluteolytic effects [169–171]. Pituitary-derived prolactin is also key to luteal function. In addition to preventing the catabolism of progesterone and increasing its production [172–177], it also enhances cholesterol uptake and maintains its stores [178–181]. Furthermore, prolactin enhances estradiol/estrogen receptor synthesis [182–184]. This latter role accounts for the angiogenic effects of prolactin, which are believed to be in part mediated through estradiol [162, 185]. In turn, estradiol stimulates progesterone biosynthesis, vascularization and hypertrophy of the corpus luteum, as well as enhancing cholesterol synthesis, circulatory uptake, and intracellular mobilization [186–191]. The corpus luteum also produces androgens, principally androstenedione. In addition to being substrates for estrogen synthesis, these can stimulate progesterone production, thereby also having antiapoptotic effects on luteal cells [162, 192–194]. There is also some evidence that LH is involved in luteal function, where it participates in the stimulation and maintenance of progesterone production by prolactin-primed luteal cells through the intermediary of estradiol [162].

The subsequent lifespan of the corpus luteum is governed by whether fertilization and preimplantation development occur. If the released oocyte is fertilized, it will have completed its development into a late morula/early blastocyst by day 4 post-conception. By this stage, differentiation of the embryonic lineages will have commenced. Of these, the trophectoderm (which will ultimately give rise to the fetal placental components) actively secretes the LH-related peptide β-hCG by days 8–12 of pregnancy, which rescues the corpus luteum by providing a direct luteotrophic signal and prevents the onset of $PGF_{2\alpha}$-induced luteolysis. In response to this, the corpus luteum persists and secretes progesterone until this role is later taken over by the fetoplacental unit in the second trimester of pregnancy [149]. However, in cases where fertilization does not occur or preimplantation development is unsuccessful, this signaling process fails: lutein cells shrink, their nuclei become pyknotic, and luteal cells are progressively replaced by connective tissue as the corpus luteum degenerates to form a corpus albicans.

There is an inbuilt safety mechanism to premature activation of this pathway: the oocyte produces BMP-15 and GDF-9, which prevent luteinization until after ovulation has occurred [195, 196]. These effects are additionally regulated by activin A, an autocrine GC factor which either promotes or inhibits follicular cell steroidogenesis depending upon their degree of differentiation and maturation [197].

The Corpus Albicans

Regression of the corpus luteum is associated with marked tissue remodeling and fibrosis,

resulting in the formation of a scar which is eventually reabsorbed into the surrounding ovarian stroma: the corpus albicans. The onset of these histological changes is heralded by the functional regression of the corpus luteum and an associated marked decrease in progesterone biosynthesis [149]. Much of the data available are drawn from rodent and ruminant studies and whether these findings are broadly applicable to the human ovary is less clear. Nevertheless, both $PGF_{2\alpha}$ and LH appear to be significant drivers in this process. In most species, $PGF_{2\alpha}$ is largely produced by the uterus, although it has been proposed that its pulsatile release favors production by the corpus luteum itself, thereby amplifying luteolytic signaling [162, 198–200]. By contrast, luteolysis in primates is not mediated by uterine $PGF_{2\alpha}$ and appears to depend entirely on luteal production [149, 201]. Rather than inhibiting progesterone biosynthesis *per se*, $PGF_{2\alpha}$ appears instead to promote its metabolism to 20α-dihydroprogesterone through an increase in 20α-HSD levels [162]. In addition, $PGF_{2\alpha}$ has also been suggested to impair progesterone production by reducing ovarian cholesterol transport and suppressing luteal estradiol production [162, 202]. LH also appears to have a paradoxical role in favoring luteolysis, although the means by which this is achieved are unclear. In addition to stimulating 20α-HSD activity [203], LH has been shown to inhibit the conversion of pregnenolone to progesterone in cultured rodent luteal cells *in vitro* [204], thereby providing a 20α-HSD-independent mechanism for reducing progesterone biosynthesis.

As functional regression of the corpus luteum is under way, structural involution becomes easier to appreciate histologically. Luteal cell death is accompanied by a loss of the gland's vascular supply as this becomes fibrotic and regresses. Rodent models have suggested that this process may be initiated by apoptosis of vascular endothelial cells, which leads to ischemia and subsequent necrosis of steroidogenic luteal cells [162, 205]. The withdrawal of progesterone also appears to facilitate additional involutional mechanisms such as $PGF_{2\alpha}$-dependent luteal cell autophagocytosis and various prolactin-induced processes. These include lymphocyte Fas-mediated apoptosis, monocyte/macrophage-driven clearance, MMP-1 and MMP-2 extracellular matrix breakdown, and ascorbate depletion (which promotes oxidative damage to luteal cells) [171, 206–212].

Gestation, Lactation, and Under-/Overnutrition: A Pregnant Pause in Ovarian Function

The sensitivity of the ovary to various systemic cues means that its endocrine and reproductive functions can respond to a multitude of host and environmental variables. Perhaps the most obvious of these are the effects of pregnancy, lactation, and nutrition. Given the central role played by the ovary in the establishment and maintenance of pregnancy, reproductive energy balance has imposed a control of ovarian function in response to the environmental nutrient supply. The hypothalamic-pituitary-ovarian axis is therefore geared to sense negative energy balance and accordingly impose a transient limitation on fertility whenever nutrient availability is insufficient to support pregnancy.

The mechanisms governing the failure to support ongoing folliculogenesis during both pregnancy and lactation both center around GnRH. Experimental administration of exogenous GnRH to women in the first and second trimesters of pregnancy has previously been shown to fail to elicit pituitary FSH or LH release, pointing to a pituitary refractoriness to stimulation [213, 214]. Despite this, the gonadal response to gonadotropins appears to remain intact during gestation, as suggested by the observation that ovulation can be induced by exogenous human menopausal gonadotropin/hCG administration in Rhesus monkeys during midpregnancy [215].

Lactation or, more specifically, the suckling stimulus associated with breastfeeding also affects ovarian function and results in amenorrhea. This appears to have evolved as an adaptation to provide the optimal birth spacing for survival of children rather than supporting a role for nutritional status [216–218]. In particular, both the frequency and duration of breastfeeds (especially at night) are crucial in this regard [218]. Suckling stimulates the local release of

β-endorphins, although there is some debate as to whether this definitely plays an active role in disrupting the normal pulsatile pattern of hypothalamic GnRH secretion, thereby reducing pituitary LH secretion and impairing follicular development [217, 219]. Regardless, the normal positive feedback effect of estrogen on LH release is abolished, and estradiol exerts an enhanced negative feedback effect on both LH and FSH. Thus, although FSH secretion returns to its normal cyclic pattern early in lactation, any follicle that starts to develop and secrete estradiol will inhibit further LH release, thereby preventing ovulation while suckling continues [216]. The suckling stimulus may also affect GnRH secretion by affecting prolactin, opioid, and dopaminergic tone in the hypothalamus [217, 219]. Again, an involvement for β-endorphin has been proposed: this suppresses dopamine secretion, thereby stimulating prolactin secretion, but this notion remains contentious [216, 219]. Other local mechanisms have also been implicated, such as impaired folliculogenesis secondary to reduced ovarian insulin and insulin-like growth factor (IGF)-1 concentrations [220]. When suckling declines, the pulsatile pattern of LH secretion returns to normal and sensitivity to estrogen negative feedback declines such that follicle growth and ovulation can resume [216]. The resumption of fertility proceeds in two parts: an initial phase of amenorrhea in which ovarian follicle growth is suppressed or attenuated followed by a resumption of menstrual cycles often associated with inadequate luteal function contributing to the reduced fertility of lactating menstruating women [216].

A deficit in caloric intake is well recognized to affect reproductive function and has, in particular, been noted in women suffering from anorexia nervosa or in those following extreme exercise regimens who exhibit impaired LH release as a result of decreased GnRH pulse amplitude and/or frequency [221, 222]. Predictably, these changes therefore impact on follicular development and impair gonadal sex steroid biosynthesis [223]. Much of this hypothalamic-pituitary-ovarian axis dysfunction is attributable to the input of peripheral systems which gauge nutritional status. Chief among these are insulin and adipocyte leptin, which provide an indication of carbohydrate provision and fat stores, although IGF-1 and direct signaling by glucose and lipids have also been implicated [224–227]. During energy deficit, the circulatory levels of these mediators fall markedly, accounting for the suppression of GnRH activity induced by negative energy balance. Many of these mediators also target the ovary directly. Among these, while leptin can cause dose-dependent inhibition of FSH and IGF-1-induced GC estradiol biosynthesis *in vitro*, it can also inhibit LH-stimulated androstenedione production by TCs and modulate perifollicular blood flow [228–230]. As such, it is perhaps unsurprising that leptin levels impact on follicular development, oocyte maturation, and ovulation [231].

Similarly, murine models indicate that excess energy intake also perturbs GnRH pulsatility and LH release. Fasting hyperglycemia, hyperinsulinemia, and hyperleptinemia accompanied by insulin and/or leptin resistance affect hypothalamic GnRH gene expression and result in elevated LH levels which, in turn, may affect the ability of steroid hormones to exert negative feedback on GnRH secretion [232–236]. Hypersecretion of LH and an increased LH:FSH ratio have been demonstrated to be unfavorable for folliculogenesis and oocyte maturation; both conditions can be observed in obese infertile patients [237–241]. Insulin also acts directly on the ovary, stimulating ovarian steroidogenesis in TCs and GCs, and enhances the stimulatory effect of LH through upregulation of its receptor [232]. These perturbations may also skew the type of sex steroid produced: obesity can increase ovarian/adrenal androgen production, thereby affecting GnRH/LH secretion, a mechanism proposed to account for the premature onset of puberty in girls [242]. Furthermore, an increased adipose tissue load with its associated macrophage infiltrate results in a greater systemic release of inflammatory mediators such as TNF-α and IL-6 [243–246]. Chronic cytokine stimulation of the hypothalamus has been proposed to be an additional mechanism by which central insulin and leptin resistance are acquired, as well as being responsible for inhibiting gonadotropin secretion, ovu-

lation, steroidogenesis, and luteal regression [135, 245–250].

The integration of nutritional sensing and reproductive function is attributable to interacting neuropeptide systems such as kisspeptin, galanin-like peptide, neuropeptide Y, pro-opiomelanocortin/α-melanocyte stimulating hormone, orexin, and nesfatin-1 (reviewed by Acosta-Martínez [227]). Importantly, many of the neurons that synthesize these neuropeptides co-express insulin IGF-1 receptors and/or leptin receptors, and their expression is regulated both by metabolic hormones and sex steroids such that their effects depend on the hormonal milieu (i.e., the presence of estrogens), developmental stage (i.e., prepubertal vs. adult), and energy status (i.e., fasted vs. normally fed) [227, 251–253]. Adipose tissue plays a central role in this regard through its production of neuropeptides, cytokines, adiponectin, leptin, and IGF-1 and IGF-2 [254, 255]. Leptin targets hypothalamic neuropeptide Y, pro-opiomelanocortin, and kisspeptin, through which it affects GnRH pulsatility and subsequent pituitary LH release and, in turn, follicular steroidogenesis [255]. Other peripheral indicators of metabolic status include insulin and ghrelin, whose concerted actions are supplemented by central detector systems based on neuropeptide Y, melanocortin, and orexin production, which also target GnRH release [256]. However, adipose tissue also provides a site for peripheral steroid production, particularly the conversion of androgens to estrogens, as well as sequestering lipophilic steroid hormones, thereby altering their delivery to target organs [224, 226, 257]. These adverse effects are compounded by decreases in sex-hormone-binding globulins (due to reduced hepatic biosynthesis in response to hyperinsulinemia) which impacts on overall steroid bioavailability and feedback on the hypothalamic-pituitary-ovarian axis [224, 226, 258, 259].

The Menopause

The onset of the menopause is heralded by the climacteric, a decade or so long period of irregular menstrual cyclicity akin to that first seen around the time of puberty. The hypothalamus-pituitary-ovarian hormonal axis weakens: on one hand, there is a decrease in pituitary and hypothalamic responsiveness to circulatory estrogens; on the other, ovarian responsiveness to pituitary gonadotropins also falls. This loss in negative feedback leads to a dramatic increase in both LH and FSH levels [260]. Together with the decreasing number of follicles associated with the menopause, the ovary's volume decreases and its cortex becomes thin, irregular, and gyrated, such that postmenopausal ovaries are largely composed of stromal cells [261, 262]. This acceleration of follicle loss is matched by an exponential loss in the number of oocytes (whose quality/viability also declines) as women enter the menopausal transition [263, 264].

Functionally, however, the postmenopausal ovary retains an androgenic endocrine function, producing 40 and 20 % of the body's total amount of testosterone and androstenedione, respectively [265]. Stromal atrophy commonly accompanies that of the cortex following the onset of the menopause, although it also contributes to the ovarian endocrine function. Indeed, non-neoplastic stromal hyperplasia with bilateral ovarian enlargement can occur postmenopausally, where it results in increased levels of both androgens and estrogens and, perhaps unsurprisingly, with virilism and endometrial polyps, hyperplasia, and carcinoma [266]. Typically, however, after an initial FSH-dependent increase in estrogen levels in the early stages of menopause, secretion subsequently declines and ovulation ceases [267]. The withdrawal of estrogen has a plethora of physiological effects, including atrophy of the reproductive organs, vasomotor changes (responsible for the well-recognized hot flushes and night sweats), increased bone catabolism (due to a loss in the anti-parathyroid effect of estrogens), and an array of behavioral changes (e.g., depression) [268]. Other hormonal changes also accompany the transition to the menopause: falling levels of inhibin B (produced by growing follicle GCs) and anti-Müllerian hormone (produced by all post-primordial follicles) are harbingers of a decline in ovarian function [269, 270].

Ovarian Functional Disorders

The ovary is affected by a spectrum of developmental and functional disorders, many of which form a significant part of the routine gynecological practice relating to infertility/assisted conception. In addition, the ovary is also the site or origin for various tumors, most notably those of epithelial origin, germ cell tumors, and sex cord stromal tumors, although these are discussed more extensively in other chapters, to which the reader is referred.

Premature Ovarian Failure

Premature ovarian failure (POF) is a good example of the failure of one or more aspects of ovarian function and highlights the multiple roles played by the ovary in maintaining normal physiology. This heterogeneous disorder affects ~1 % of women by age 40 years and presents as a broad spectrum of symptoms ranging from primary amenorrhea associated with absent menarche (half of cases being due to ovarian dysgenesis) through to secondary (i.e., postpubertal) amenorrhea, which features premature follicle depletion or arrested folliculogenesis [271, 272]. The causes of POF are multiple: iatrogenic (surgery, chemo/radiotherapy), chromosomal (Turner and fragile X syndromes), autoimmune, infectious (herpes zoster, cytomegalovirus), monogenic (e.g., FSH/LH receptor and BMP-15 mutations), or idiopathic [273].

X chromosome abnormalities such as Turner syndrome account for the majority of primary amenorrhea cases associated with ovarian dysgenesis, and, predictably, these feature an absence of both pubertal development and its associated growth spurt [173, 274, 275]. Ultrasound investigations frequently reveal streak ovaries and uterine hypoplasia although some degree of follicular and pubertal development can exist in some monogenic defects such as FSH/LH receptor mutations as well as cases of Turner syndrome associated with mosaic karyotypes or structural chromosomal abnormalities [276, 277]. However, most cases of POF occur postpubertally and recapitulate the symptoms of the climacteric/menopause (palpitations, heat intolerance, hot flushes, mood changes, fatigue) and may either occur abruptly or develop gradually over a period of years [273]. In addition to the obvious impact on fertility, the associated protracted estrogen deficiency leaves sufferers vulnerable to early onset osteopenia/osteoporosis as well as increasing the risk of Alzheimer's dementia and cardiovascular disease if left untreated.

As might be expected, both primary and secondary POF feature low levels of ovarian steroid and peptide hormones (estrogens, inhibins). By contrast, gonadotropin levels are typically elevated: FSH usually predominates, except in rare cases of LH receptor mutations [273]. These endocrine changes have been targeted as promising circulatory biomarkers of ovarian reserve, especially anti-Müllerian hormone and inhibin B, whose falling levels have been associated with POF [278, 279].

Polycystic Ovary Syndrome (PCOS)

PCOS affects approximately 5–10 % of women of reproductive age and, from a gynecological perspective, is typically associated with oligo-/amenorrhea, hyperandrogenism, and the presence of multiple follicular cysts [280–283]. In turn, it is a significant cause of infertility, endometrial hyperplasia, and, less commonly, premenopausal endometrial adenocarcinoma. The syndrome also features a wide range of endocrine and metabolic abnormalities leading to insulin resistance, obesity, hypertension, dyslipidemia, and hyperhomocysteinemia [284]. The first of these is a key feature in the pathogenesis of PCOS: on one hand, insulin acts in concert with LH to promote ovarian androgen production; on the other, it depresses hepatic sex-hormone-binding globulin production, thereby resulting in higher levels of free testosterone [232, 285–287]. These effects aggravate the underlying increased GnRH pulsatility which results in higher LH levels combined with a relative deficiency of FSH, accounting for the elevated LH:FSH ratios characteristic of the syndrome [31, 288–290]. It has been proposed that this reflects a failure of suppression of GnRH pulsatility due to a primary hypothalamic dysfunction (e.g., perturbed noradrenergic or γ-aminobutyric acid pathways, which remain mechanistically unconvincing), the response to

an abnormal hormonal environment, or, possibly, a combination of both [31].

The abnormal steroidal milieu of PCOS has several possible origins. On one hand, prolonged oligo-/anovulation results in affected women seldom experiencing regular postovulatory increases in progesterone which contributes to maintaining persistently rapid GnRH pulsatility, a phenomenon further aggravated by a reduced sensitivity of the GnRH pulse generator to progesterone negative feedback [31, 291]. This reduced sensitivity is believed to be secondary to hyperandrogenemia, rather than being a primary hypothalamic defect, as highlighted by the fact that sensitivity can be restored by androgen receptor blockade [292]. Although it has been proposed that this neuroendocrine anomaly could trace its origins to prenatal androgenization (consistent with elevated gestational androgen levels in PCOS and the fact that the disorder clusters in families in the absence of well-defined genetic causes), whether these variables are causative or coincidental remains to be proven [31, 282, 293, 294]. Regardless of the mechanism involved, the rapid GnRH pulsatility in PCOS and its resultant increased LH:FSH ratio translate into excessive ovarian androgen biosynthesis and ovulatory dysfunction, thereby establishing a self-perpetuating cycle of endocrine dysfunction.

PCOS has also been associated with a decreased sensitivity of the hypothalamic-pituitary-ovarian axis to insulin as part of the systemic resistance noted in many sufferers [227, 245, 295]. The resultant loss of the anti-inflammatory effects of insulin in part accounts for the low-grade inflammation characteristic of the syndrome [227, 296–298], although causal relationships are unclear. Indeed, as an adipocyte-derived inflammatory cytokine, TNF-α itself is thought to induce insulin resistance by impairing insulin receptor signaling, although this mechanism may also involve other mediators such as IL-6, IL-18, and C-reactive protein [245, 298–301]. The likely involvement of cytokines in the pathophysiology of the syndrome is supported by the anti-inflammatory effects of metformin which, while improving reproductive function in women with PCOS, has been shown to reduce systemic CRP levels and to inhibit cytokine production from an array of cell types [227, 295]. Interestingly, TNF-α, IL-6, and insulin can affect steroid production (e.g., through a suppression of GC aromatase) and may in part account for the sustained ovarian androgen synthesis production in PCOS [245, 287, 302].

Other theories instead focus on a follicular basis to the disorder. PCOS follicles are thought to undergo developmental arrest when they reach a diameter of 4–7 mm, thereby accounting for the accumulation of large numbers of small antral follicles beneath the tunica albuginea, whose theca interstitial cells exhibit abnormally high levels of androgen biosynthesis [303, 304]. Both human and animal studies suggest that many of these problems can be traced to a delayed and reduced expression of GDF-9 in primary oocytes during their growth and differentiation phase which, in turn, perturb follicle growth, inhibit kit ligand expression, increase inhibin A production, and, overall, inhibit FSH-dependent GC responses such as estrogen, progesterone, and LH receptor expression [195, 305–307]. These changes are accompanied by hyperplasia and hypervascularity of the theca interna and stroma associated with androgen production which are believed to result from aberrant expression profiles of components of the VEGF system [308].

Endometriosis

Endometriosis remains a leading gynecological disorder believed to affect up to 10 % of premenopausal women, with an average age at diagnosis of 29–30 years [309, 310]. It features the presence of functional endometrial glands and stroma outside the uterine corpus, affecting sites such as the fallopian tubes, ovaries, bowel, uterosacral ligaments, and peritoneum. Endometriosis can be asymptomatic but typically presents as cyclical/chronic pelvic or abdominal pain, secondary dysmenorrhea, menorrhagia, rectal bleeding, dyspareunia, and/or subfertility [311]. The pathophysiology of endometriosis is complex and multifactorial, involving progesterone resistance, a local estrogen-rich environment, and a marked inflammatory response [312].

Over the last three decades, there has been a mounting body of evidence implicating endometriosis in ovarian pathophysiology. Early studies reported an increased prevalence of the disorder in association with subfertility following laparoscopic evaluation (21–47 % vs. 5 % in controls) [313–315]. The cause for this association is multifaceted as the entire spectrum of ovarian function and systemic gonadotropin profiles are affected. In brief, endometriosis has been associated with a reduced follicular growth rate, prolonged follicular phase, and reduced preovulatory follicle size across a range of studies [316–319]. Indeed, the prevalence of abnormal follicular development has been reported to affect as many 40 % of cycles [320]. In turn, these perturbations in ovarian dynamics have also been linked to a deregulated/impaired LH surge, reduced preovulatory serum estrogen levels, and anomalous early luteal phase profiles of both estrogen and progesterone [316, 318, 319, 321]. Although there has been some skepticism as regards the impact of endometriosis on ovarian steroidogenic activity [322], recent investigations suggest that inflammatory perturbations relating to increased PGE_2 concentrations may be responsible for the circulatory imbalances in estrogen and progesterone through aberrant regulation of the expression of the enzymes responsible for ovarian steroid biosynthesis [323]. Thus, endometriosis appears to affect both the functionality of the ovary and that of the hypothalamic-pituitary-ovarian axis in up to 82 % of cycles studied [321, 322].

The impact on oocyte viability and reproductive function is nevertheless clear. Endometriosis reduces *in vitro* fertilization rates by 25 % and has adverse effects on preimplantation development, implantation, and overall pregnancy rates [322]. While some of these effects are undoubtedly attributable to perturbations in follicular growth and endocrine disruption, the pathophysiological scenario has also been suggested to implicate local ovarian inflammatory dysfunctions associated with increased cytokine and prostaglandin levels [323, 324].

Conclusion

The ovary is a dynamic organ whose interrelated endocrine and reproductive blueprints are established during intrauterine life and hinge on successive waves of follicle development throughout the four decades of its functional lifespan. Moreover, the ovaries do not operate in isolation: much of the cyclical changes in steroid hormone profiles are an intrinsic part of the ovarian cycle-dependent positive and negative feedback mechanisms which regulate the hypothalamic-pituitary-ovarian axis.

The functional units within the ovary are its follicles whose development is orchestrated by an extensive network of cytokines and growth factors which operate as paracrine mediators across the intrafollicular microenvironment in response to the prevailing gonadotropin and steroid milieu. In addition to these systemic cues, the ovary is sensitive to conceptus-derived β-hCG which prolongs the life of the corpus luteum and ensures the successful establishment of pregnancy. Although the complexity of the control of ovarian function falls little short of being a wonder of evolutionary bioengineering, it has the drawback of making it vulnerable to deregulation, resulting in a number of functional disorders associated with endocrine dysfunction and infertility.

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Nonneoplastic Disorders of the Ovary

2

Judith N. Bulmer

Abstract

Histopathologists frequently receive oophorectomy specimens for a variety of reasons other than assessment of an ovarian neoplasm. Recognition of various incidental findings and nonneoplastic lesions in the ovary is therefore an important part of daily practice for histopathologists. Functional cysts can arise from follicular structures and lead to clinical symptoms, while stromal lesions can be associated with hormonal disturbances and secondary endometrial pathology. Ovarian inflammation can occur as part of pelvic inflammatory disease, in various viral and parasitic infections, as well as in noninfectious inflammatory conditions. Endometriosis is commonly encountered in the ovary, with variable features including formation of large endometriotic cysts. It is important to be aware and recognize a range of ovarian lesions that occur specifically in pregnancy to avoid unnecessary treatment. Ovarian torsion is a not uncommon gynecological emergency, and various miscellaneous lesions are frequently encountered during routine histopathological examination of the ovary. This chapter provides a summary of the main nonneoplastic conditions of the ovary that are likely to be encountered by histopathologists.

Introduction

The ovaries are received for histopathological examination for many reasons apart from their removal for assessment of an ovarian neoplasm. Oophorectomy is performed as part of staging for a range of other gynecological malignancies. Bilateral salpingo-oophorectomy may be performed in both premenopausal and postmenopausal women at the time of hysterectomy for benign conditions, and normal physiological findings and incidental pathological lesions can be detected. Women with a family history of ovarian cancer may undergo prophylactic bilateral salpingo-oophorectomy as prophylaxis. Hence, understanding of normal ovarian histology and nonneoplastic ovarian histopathology is

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essential for both general and specialist gynecological histopathologists on a daily basis. This chapter considers the main nonneoplastic conditions encountered in histopathological examination of the ovary.

Follicular and Stromal Lesions

Cystic Follicles and Follicular Cysts

Cystic Follicles

Follicular development occurs throughout reproductive life and, since follicle development does not occur within a single cycle, the various stages of follicle development can be observed in the ovaries from women of reproductive age. Cystic follicles are within the range of normal follicular development. They arise in reproductive age women when Graafian follicles do not rupture to release the ovum and eventually undergo resorption. Cystic follicles can be solitary or multiple and macroscopically appear as smooth-walled cortical cysts, usually <1 cm diameter, that contain watery fluid. By definition once cystic follicles reach 3 cm diameter, they are termed follicle cysts. Cystic follicles are usually lined by inner granulosa cells and outer theca cells which show varying luteinization. Some may resemble corpora lutea and are lined by luteinized granulosa cells and theca cells.

Follicle Cysts

Etiology and Pathogenesis: Follicle cysts usually present in nonpregnant women of reproductive age, particularly around menarche and in the perimenopause, but rarely can occur in newborns and childhood [1] and in postmenopausal women [2]. The etiology is likely to reflect disordered function of the pituitary-ovarian axis. Non-luteinized follicle cysts secrete estrogen and arise by excessive ovarian stimulation, either by endogenous FSH or exogenous ovulation-inducing agents. Granulosa lutein cysts secrete progesterone and may arise from failure of follicular rupture at ovulation. Theca-lutein cysts show luteinization of the theca interna and secrete androgens; they develop with prolonged exposure to LH or βhCG such as in polycystic ovarian syndrome, pregnancy, or ovarian hyperstimulation syndrome.

Clinical Features: Cystic follicles are usually asymptomatic but may present with mild vague pelvic pain. Non-luteinized follicle cysts are also often an incidental finding but sometimes present with menstrual irregularities due to estrogen production. Granulosa lutein cysts secrete progesterone but rarely at sufficient levels to disrupt the menstrual cycle [3]. Occasionally, follicle cysts may rupture and cause acute abdominal pain and hemoperitoneum [4] or present as an ovarian mass. Ultrasound is usually reassuring. Cyst aspiration with cytological assessment and measurement of estradiol has been reported as a diagnostic approach [5, 6], but initial treatment is usually by observation rather than aspiration due to the theoretical potential of dissemination of malignant cells. A randomized controlled trial of cyst aspiration compared with observation in 269 women aged 14–81 years reported no difference in the resolution rate at 6 months of ultrasound-detected simple ovarian cysts after cyst aspiration (46 %) compared with observation (44.6 %) [7]. Rarely follicular cysts are seen in neonates and give rise to isosexual pseudoprecocity; these usually regress within the first 4 months after birth.

Histopathological Appearances: By definition, follicle cysts are >3 cm in diameter and usually do not exceed 10 cm. Macroscopically they have a smooth surface and thin walls and contain watery fluid. Microscopically follicle cysts typically show an inner lining of several layers of granulosa cells, which may be luteinized, and an outer lining of theca cells (Fig. 2.1a); the two layers can be delineated with a reticulin stain. During involution, the lining becomes thin (Fig. 2.1b) and ultimately may be denuded, especially the granulosa cell layer, in which case the differential diagnosis is with a simple indeterminate cyst. Depending on the extent of luteinization, follicle cysts may be non-luteinized or show luteinization of granulosa cells (granulosa lutein cyst) or theca interna cells (theca-lutein cysts).

Differential Diagnosis: Rarely the differential diagnosis of follicle cysts is with a cystic granulosa cell tumor; these are usually lined by plump

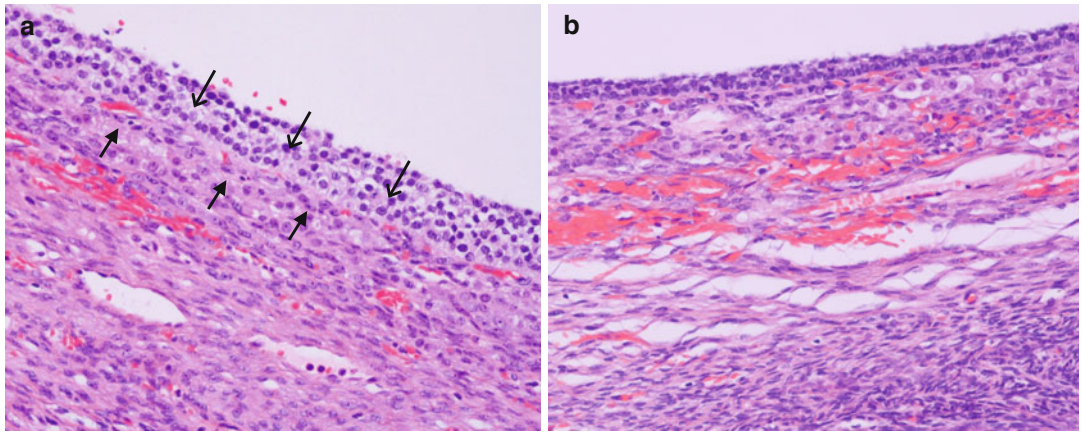

Fig. 2.1 (**a**) Follicle cyst lined by inner layer of granulosa cells (*open arrows*) and outer luteinized theca interna cells (*closed arrows*). (**b**) Follicle cyst showing a thin inner layer of granulosa cells suggesting involution

granulosa cells that are irregularly arranged and are usually multicystic. A possible diagnostic pitfall arises by crosscutting of cystic follicles and misinterpretation of the plump and mitotically active theca cells as a stromal neoplasm.

Corpus Luteum and Corpus Luteum Cysts

In the normal menstrual cycle, corpora lutea are the result of ruptured follicles. Corpus luteum cysts usually occur in women of reproductive age but can rarely occur in neonates [8] or after sporadic ovulation in postmenopausal women. Macroscopically they are recognized by their yellow appearance when recent and as white nodules when degenerating. They arise when excessive hemorrhage delays involution of the corpus luteum after ovulation and may be cystic or hemorrhagic.

By convention, a corpus luteum cyst is generally designated as a cystic corpus luteum that is >3 cm in diameter. Most are asymptomatic, but they may present with menstrual abnormalities or amenorrhea and occasionally rupture giving acute abdominal symptoms [4]. They may also be detected on ultrasound examination in normal early pregnancy. Macroscopically, in common with the smaller cystic corpus luteum, they are yellow in appearance and often filled with altered blood or clear fluid. The size may reach 10 cm diameter. Microscopically corpus luteum cysts are lined by a thick layer of luteinized granulosa cells with an outer layer of smaller luteinized theca interna cells; there may also be an inner layer of connective tissue (Fig. 2.2a). There is a prominent zone of vascularization resulting from the growth of blood vessels growing from the theca into the collapsed granulosa cell layer. The granulosa cells and theca-lutein cells are smaller than those seen in a fresh corpus luteum; nuclei are small and hyperchromatic, and mitoses are not seen. As involution proceeds, there are smaller islands of lutein cells and increasing fibrosis (Fig. 2.2b).

The differential diagnosis includes luteinized follicular cysts which are less likely to be hemorrhagic and also lack the vascularized zone that is seen in corpus luteum cysts. Some follicle-derived cysts may be difficult to classify. Endometriotic cysts may be indistinguishable macroscopically, but the diagnosis is clear on microscopic examination with the detection of endometrial glands and stroma.

Corpus Albicans Cysts

A corpus albicans is usually a solid hyalinized scar but occasionally is cystic with a central cavity that contains clear fluid. These cysts are usually <1 cm diameter and are lined by a band of hyalinized fibrous tissue. They are asymptomatic.

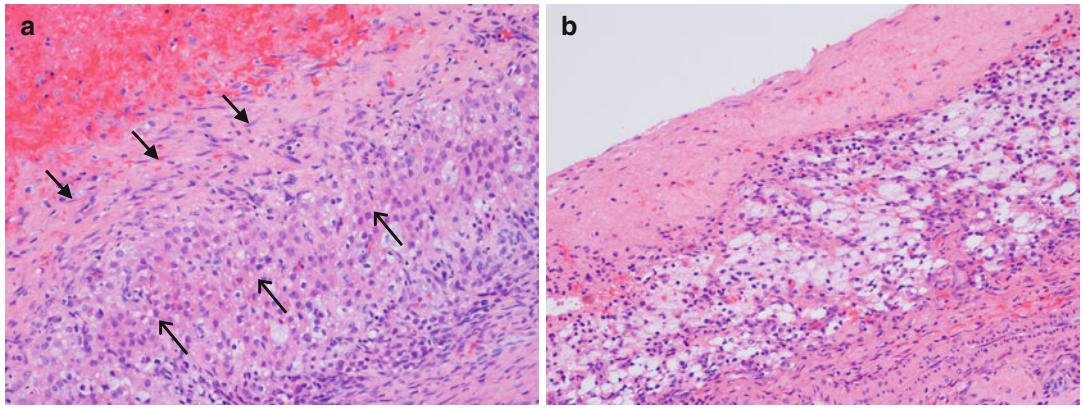

Fig. 2.2 (**a**) Early corpus luteum cyst with organizing hematoma and lining of fibrous tissue (*closed arrows*) and luteinized cells (*open arrows*). (**b**) Late corpus luteum cyst with an inner layer of fibrous tissue and vacuolated involuting luteinized cells

Polycystic Ovarian Syndrome

Introduction: Polycystic ovarian syndrome (PCOS; previously Stein-Leventhal syndrome) is a common cause of infertility. It is the most common endocrine disorder in women, with a prevalence of 6–10 % based on US National Institutes of Health criteria and as high as 15 % based on the broader Rotterdam criteria [9, 10]. Definitions of PCOS vary and more recently have been based on clinical features, rather than the morphological features of polycystic ovaries. Definitions take into account features of oligo- or anovulation; clinical or biochemical hyperandrogenism, for which other etiologies have been excluded; and polycystic ovaries, diagnosed by ultrasound. A 2003 consensus workshop sponsored by ESHRE/ASRM in Rotterdam [11, 12] agreed PCOS to be present if any two of the following three criteria were met, provided other causes were excluded: oligo-ovulation and/or anovulation, excess androgen activity, and polycystic ovaries. Other common features observed in PCOS are obesity and insulin resistance, with or without associated diabetes, but these are not part of the diagnostic criteria. PCOS represents a spectrum of features with some cases having less severe manifestations, and many women with polycystic ovaries have less pronounced metabolic alterations. Women with amenorrhea, androgenic manifestations, and enlarged polycystic ovaries represent the severe end of the spectrum.

Pathogenesis: The pathogenesis of PCOS is not clear. The syndrome is often familial, suggesting involvement of hereditary factors, but the specific mode of inheritance has not been determined [13, 14]. Multiple biochemical pathways are implicated in the pathogenesis of PCOS, and hence a wide range of genes have been investigated, including genes of steroid hormone metabolism, gonadotropin release and action, insulin secretion and action, adipose tissue metabolism and genes encoding inflammatory cytokines. Nevertheless, to date, no clear gene associations have been detected, and none of these genes appear to play a key role in the etiology and pathogenesis of PCOS. It is likely that environmental and genetic factors interact and that PCOS is inherited as a complex, polygenic trait [15, 16]. The determination of the genetic factors that predispose to PCOS is hampered by the low fecundity of affected females, the lack of a male phenotype, the absence of a good animal model, and until recently the lack of consensus on the diagnostic criteria.

The pathophysiology of PCOS is complex and not completely understood. It is likely that many interlinked mechanisms play a role in the initiation and/or perpetuation of PCOS [17, 18]. Some patients with PCOS have the HAIR-AN syndrome of hyperandrogenic-insulin-resistant-acanthosis nigricans. This syndrome can be separated into insulin-resistant and nonresistant

types; the insulin-resistant type has PCOS and increased LH, whereas the nonresistant type has stromal hyperthecosis and near-normal LH levels [19]. The HAIR-AN syndrome highlights the complexities of the links between insulin resistance, PCOS, and hyperthecosis.

Clinical Features: By definition, PCOS is not associated with underlying pituitary or adrenal pathology. It is a clinicopathologic syndrome and the presenting features vary with age. PCOS is associated with precocious puberty in children, menstrual disturbances in teenagers, and infertility and glucose intolerance in adults. Obesity exacerbates the insulin resistance and favors progression to diabetes, but whether obesity is a cause or an effect of PCOS is unclear. There is a strong association with subfertility and infertility, and there are various approaches to the management of this clinical problem [20, 21]. Many patients have increased LH levels due to increased LH pulse size and frequency, and these high LH levels lead to excessive thecal growth and hence androgen production. FSH levels are usually normal or low, and the LH:FSH ratio is >2. Women with high LH levels are at highest risk of infertility and miscarriage [22]. Hyperinsulinemia is common.

The ultrasound appearance of the ovary shows at least 10 peripheral cysts, usually <10 mm diameter. There is increased stroma and often the ovarian volume is increased. The ovarian features of PCOS are, however, variable: some women have normal-sized ovaries with minimal microcystic change.

As well as the endocrine and cardiovascular effects, women with PCOS are at increased risk of developing endometrial hyperplasia and endometrial neoplasia, with evidence supporting a 2.7-fold increased risk of endometrial cancer in women with PCOS [9, 10]. Most endometrial neoplasms are type 1 endometrioid adenocarcinoma associated with excess estrogen stimulation, and the prognosis is good.

Histopathological Findings: Wedge resection of the ovary is no longer performed, and therefore polycystic ovaries are usually encountered in routine hysterectomy specimens or very rarely when ovarian excision is necessary to control androgenic features associated with PCOS. Macroscopically, the ovaries are enlarged with multiple bluish translucencies indicating the cystic follicles. On cut section, there are multiple small cysts usually of uniform size, 4–10 mm diameter, arranged peripherally within the cortex. The capsule is usually thickened.

Microscopically there is collagenous thickening of the tunica albuginea, usually around threefold. The follicular cysts are lined by non-luteinized granulosa cells and outer layer of luteinized theca interna cells. Primordial follicles are present. Anovulatory women show minimal features of previous ovulation such as corpora lutea and corpora albicantia, but evidence of ovulation does not preclude the diagnosis. Atretic follicles are present, often surrounded by prominent luteinized theca cells. Some cases show luteinized stromal cells and luteinized hilus cells.

Differential Diagnosis: PCOS is a clinical syndrome, and the diagnosis is not made by the histopathologist. There are various conditions in the clinical differential diagnosis, including hormone-producing ovarian neoplasms. For the histopathologist, the differential diagnosis of the macroscopic and microscopic features is limited. Consideration should be given to ovarian hyperstimulation syndrome and hyperreactio luteinalis in which the ovary shows multiple theca-lutein cysts and prominent luteinized stromal cells, but the clinical situation in both these conditions should minimize confusion.

Stromal Hyperplasia

Introduction: Stromal hyperplasia is defined as an expansion of the cortex and medulla of the ovary by an excess of stromal cells [23]. Mild stromal hyperplasia is a common incidental finding in perimenopausal and postmenopausal women, with mild hyperplasia of ovarian cortical and medullary stroma found in around one third [3]. A morphometric study reported moderate to severe ovarian stromal hyperplasia in one third of women in their sixth and seventh decades, with a significant association with postmenopasual endometrial adenocarcinoma [24]. The distinction from stromal hyperthecosis is based on the

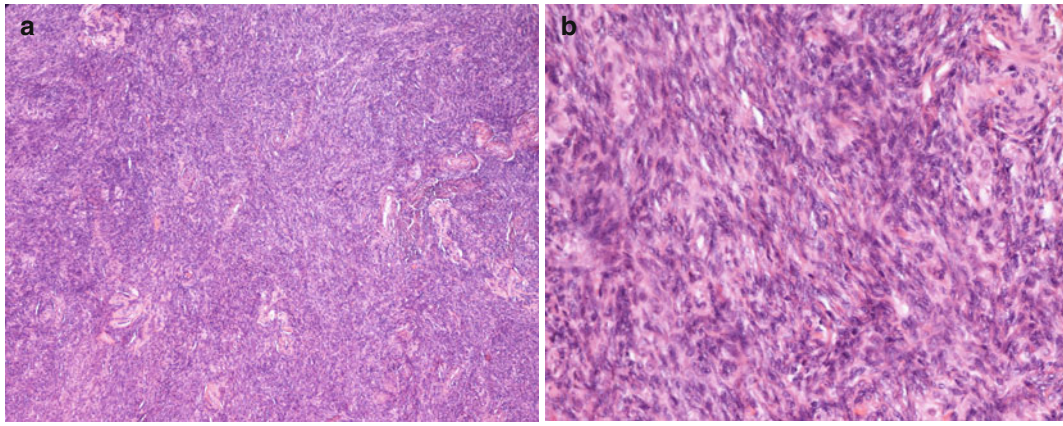

Fig. 2.3 (**a**) Cortical stromal hyperplasia characterized by whorls of spindle cells showing increased cellularity but otherwise resembling those in the normal ovarian cortex. There is loss of the normal corticomedullary interface. (**b**) Higher-power view of cortical stromal hyperplasia

presence of luteinized stromal cells, but small numbers of luteinized cells are often detected after careful searching of the cortex of ovaries with stromal hyperplasia, and these entities are likely to represent a morphological spectrum [23]. As there is little correlation of the severity of stromal hyperplasia with clinical symptoms, it has been suggested that the term stromal hyperplasia should be reserved for definite and florid cases [3]. Minimum criteria suggested for the diagnosis of ovarian stromal hyperplasia are obliteration of the normal distinction between the cortex and medulla or some nodularity to the excessive stroma.

Clinical Features: Most patients are asymptomatic, and stromal hyperplasia is detected as an incidental finding in ovaries removed for other reasons. Some cases have clinical symptoms resulting from androgen secretion by the hyperplastic stroma [25] with peripheral aromatization to estrone and potential effects on endometrium [26]. These features are less common in stromal hyperplasia than in stromal hyperthecosis.

Histopathological Findings: In stromal hyperplasia, the ovaries may be normal in size or are uniformly enlarged up to twice the normal size. The normal surface convolutions seen in postmenopausal ovarian atrophy are less obvious, and the capsular surface is smooth. The cut surface may show only a mild increase in cortical thickening or may show loss of the normal demarcation with a homogeneous tan/brown or whitish/pale yellow surface. Severe cases may show some nodularity.

Microscopically there is loss of the normal defined ovarian cortex with expansion of stromal cells that may occupy the whole ovary (Fig. 2.3a). The process may be diffuse, but more commonly there is a widespread nodular pattern particularly in the cortex. The cells are identical to those normally seen in the ovarian cortex with scanty cytoplasm and little collagen (Fig. 2.3b). Mitoses are not common. Some lesions include macrophages and have an overall "granulomatous" appearance, possibly reflecting regression of luteinized stromal cells [3].

Differential Diagnosis: The differential diagnosis is from ovarian fibroma which, in contrast with stromal hyperplasia, is usually unilateral and endometrial stromal sarcoma which shows the characteristic vascular pattern and mitoses.

Stromal Hyperthecosis

Introduction: Stromal hyperthecosis is defined as the presence of luteinized cells of thecal origin within ovarian stroma distant from the follicles. The luteinized cells may be clustered in groups or distributed singly. Some consider stromal hyperthecosis to be part of a spectrum with stromal hyperplasia,

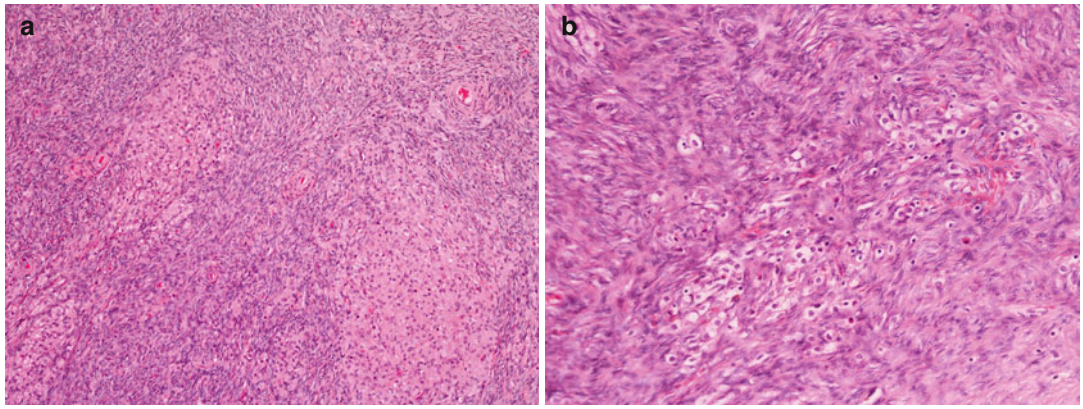

Fig. 2.4 (**a**) Stromal hyperthecosis. This low-power view shows clusters of eosinophilic luteinized stromal cells within hyperplastic ovarian cortical stroma. (**b**) Stromal hyperthecosis. These clusters of luteinized cells show clear cytoplasm and are more diffusely distributed

but other cases merge with PCOS, and there are distinctions in the clinical findings between stromal hyperthecosis and stromal hyperplasia.

Clinical Features: Although hyperthecosis may be asymptomatic in postmenopausal women, usually patients are of reproductive age and have androgenic manifestations [27, 28] and occasionally estrogenic features. Stromal hyperthecosis is one of the most common benign causes of virilization. Testosterone levels may be in the range of androgen-secreting tumors, which need to be considered in the clinical differential diagnosis. As in PCOS, patients may also have obesity, hypertension, and reduced glucose tolerance. Stromal hyperthecosis may be also seen in association with endometrial hyperplasia and endometrial carcinoma. Stromal hyperthecosis may be familial and accompanied by acanthosis nigricans, diabetes, and hyperandrogenism as part of the HAIR-AN syndrome.

Histopathological Findings: Macroscopically both ovaries are enlarged up to 8 cm diameter, although they are usually around twice the normal size. Occasionally the changes are unilateral. The cut surface shows a tan or yellow appearance, with nodularity in severe cases. Microscopically there is hyperplastic ovarian stroma admixed with luteinized cells which form small nests or are scattered singly (Fig. 2.4a). The cells are typically more numerous in the medulla and have abundant clear or eosinophilic cytoplasm, a small central nucleus, and a single conspicuous nucleolus (Fig. 2.4b). The luteinized cells can be highlighted by their immunoreactivity for inhibin and calretinin.

Differential Diagnosis: The high circulating androgen levels may require clinical exclusion of an androgen-secreting tumor. The histopathological differential is from stromal luteoma; an arbitrary size criterion of 1 cm diameter separates stromal luteoma from nodular stromal hyperthecosis.

Hilus Cell Hyperplasia

Hilus cells, also termed Leydig cells, are rounded polygonal cells with eosinophilic cytoplasm and a central vesicular nucleus. They are rarely observed in routine sections of the ovary, and therefore their detection is likely to denote hyperplasia. The definition may be difficult as the hilar cell nests are often widely separated and difficult to quantify without extensive sectioning of the ovaries [29]. Hilus cell hyperplasia is common around the menopause and in pregnancy in response to increased gonadotropin levels. In pregnancy, there may be a nodular proliferation of hilus cells with nuclear enlargement, hyperchromasia, and pleomorphism. In nonpregnant women, hilus cell hyperplasia usually arises in a background of stromal hyperplasia and hyperthecosis. Hilus cell hyperplasia is usually mild and usually not

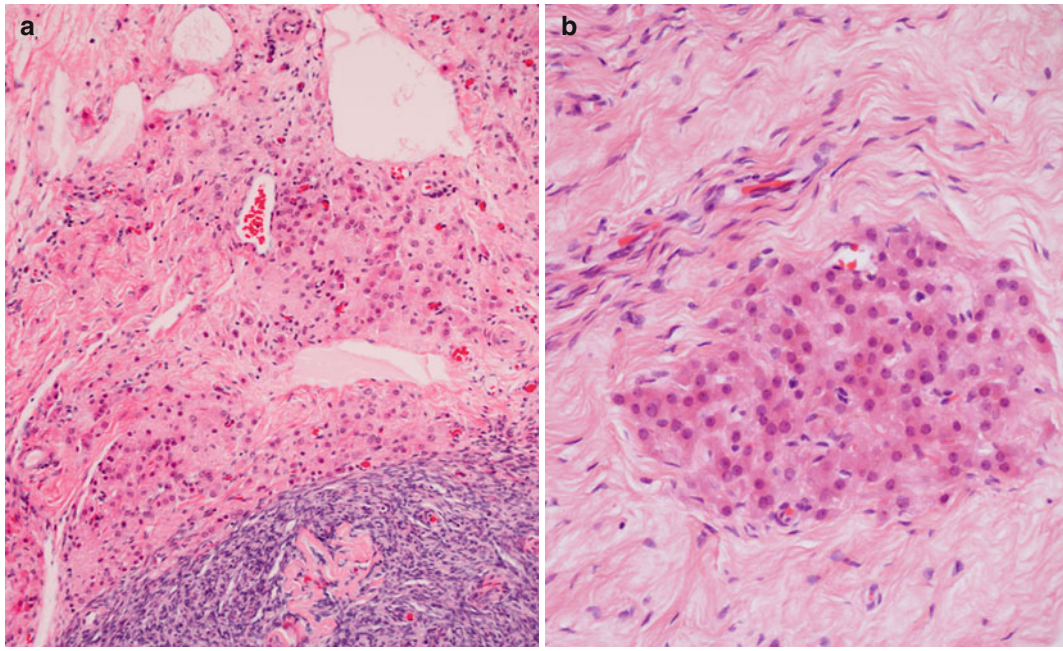

Fig. 2.5 (**a**) Hilus (Leydig) cell hyperplasia. Clusters of eosinophilic hilus cells associated with vessels in the ovarian hilus. This example was associated with stromal hyperplasia. (**b**) Hilus cell hyperplasia. Cluster of hilus cells showing typical eosinophilic cytoplasm, rounded uniform nuclei, and lipofuscin pigment

associated with endocrine disturbance, although severe cases may have virilization and high circulating androgen levels [30].

Microscopically there are clusters of typical hilus cells with eosinophilic granular to finely vacuolated cytoplasm (Fig. 2.5). Intracytoplasmic lipofuscin is often seen, and Reinke crystals may be demonstrable, although they are usually scarce. There is strong immunoreactivity for inhibin and calretinin. The distinction between hilus cell hyperplasia and a hilus cell tumor is arbitrary and based on the formation of a mass lesion [23] or size of <1 cm diameter [29, 31].

Ovarian Pathology During Pregnancy

Introduction

The cells in the ovarian cortex and hilum are highly responsive to circulating gonadotropin and steroid hormones, and this may lead to specific ovarian changes during pregnancy. The recognition of these pregnancy-associated nonneoplastic lesions is important since most will involute after pregnancy and radical surgery is not appropriate.

The corpus luteum of pregnancy is the corpus luteum that was present in the fertilized cycle. It produces progesterone and is critical for maintenance of pregnancy during the first trimester. After this time, the corpus luteum regresses and has a negligible role in pregnancy maintenance in the second and third trimesters. Features that distinguish the corpus luteum in pregnancy are enlargement of the granulosa lutein cells and the presence of cytoplasmic vacuoles (Fig. 2.6) [32]. The importance of the corpus luteum of pregnancy is the recognition of this structure as a potential cause of ovarian mass in the first trimester.

Theca-Lutein Hyperplasia of Pregnancy

Theca-lutein hyperplasia of pregnancy, nodular theca-lutein hyperplasia of pregnancy, and pregnancy luteoma can be considered to form part of

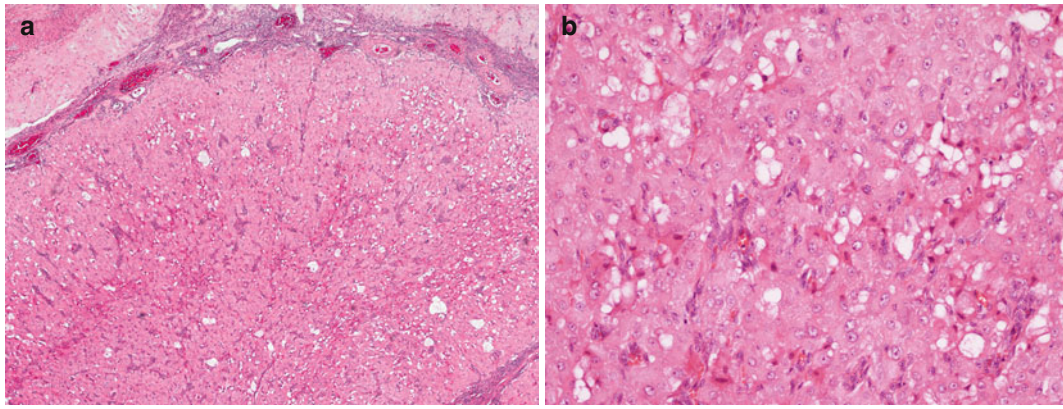

Fig. 2.6 (**a**) Corpus luteum of early pregnancy. Low-power view showing cerebriform contour and enlarged granulosa cells. (**b**) Corpus luteum of early pregnancy. High-power view showing enlarged granulosa cells and cytoplasmic vacuolation

a spectrum of changes in pregnancy, although they are often considered as separate entities. Ovaries removed at the time of delivery will show variable numbers of follicles with an attenuated granulosa cell layer and prominent often asymmetrically expanded luteinized theca. The features are similar to those seen in hyperthecosis and are due to the effects of chorionic gonadotropins on the ovary [33, 34].

Nodular Theca-Lutein Hyperplasia of Pregnancy and Pregnancy Luteoma

Introduction: These are nodular tumorlike masses of lutein cells that develop during an otherwise normal pregnancy. There is expansion of the follicular structures of theca-lutein hyperplasia and coalescence into nodules, with formation of multinodular often multicentric or bilateral nodular tumorlike masses of lutein cells [33, 34]. Although an origin from atretic follicles has been suggested [33], others consider that the luteinized cell nodules may also arise by proliferation of luteinized ovarian stromal cells [3, 29]. Pregnancy luteoma is a more extreme form of nodular theca-lutein hyperplasia but forms part of a spectrum. Pregnancy luteoma is strictly defined as the formation of an expansile solitary brown or yellow tumor that displaces the ovarian parenchyma [23]. Often the ovaries show multiple, multicentric nodules reflecting the spectrum of changes from nodular theca-lutein hyperplasia to stromal luteoma. This spectrum provides evidence that pregnancy luteomas are not true neoplasms, although it is possible that rare solitary pregnancy luteomas could represent clonal proliferation in response to the hormonal environment of pregnancy [33].

Clinical Features: Most patients with pregnancy luteoma have a history of multiple pregnancy and up to 80 % occur in black women [33, 34]. Most are asymptomatic, but they can present with abdominal pain after torsion or with tumor-like masses detected incidentally on ultrasound or at caesarean section. In some cases, pregnancy luteoma leads to virilization, and rarely increased androgen levels may affect a female fetus leading to virilization at birth [35].

Nodular theca-lutein hyperplasia and pregnancy luteoma usually present in the third trimester and involute postnatally. Although beta human chorionic gonadotropin (βhCG) is essential for the development of pregnancy luteomas, this is not the only factor since these lesions do not occur in the first trimester when βhCG levels are highest nor are they seen in association with gestational trophoblastic neoplasia [3].

Histopathological Findings: Macroscopically the ovaries show multiple or single nodules with a soft gray or tan cut surface. Pregnancy luteomas are usually 6–12 cm diameter but have been reported

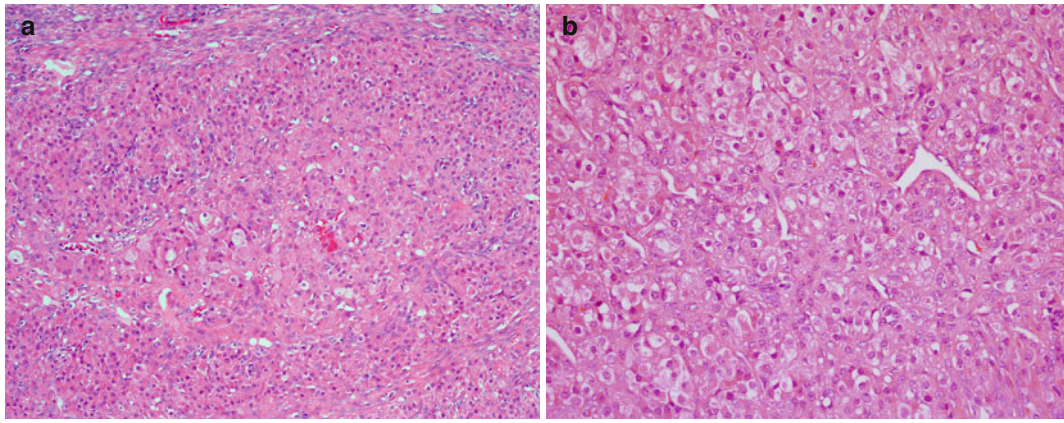

Fig. 2.7 (**a**) Nodular theca-lutein hyperplasia of pregnancy. The ovary shows nodular aggregates of luteinized cells with a well-defined interface with the surrounding stroma. (**b**) Pregnancy luteoma. Cell groups forming clusters interspersed with delicate vessels

to exceed 20 cm diameter. If the ovaries are removed in the puerperium, there may be regression and the cut surface may be soft and necrotic. Microscopically there is prominent luteinized cell proliferation with enlarged, confluent nodules of luteinized cells (Fig. 2.7a). The granulosa cells are usually inconspicuous and attenuated or involuted, and the nodules are formed of luteinized theca cells. The cytoplasm is eosinophilic, and there is a round central nucleus with a prominent nucleolus (Fig. 2.7b). There may be mild nuclear pleomorphism, and scattered mitoses may be seen but <3 per 10 high-power fields. The cells are intermediate in size between granulosa cells and theca-lutein cells. Luteinized cells may also be seen streaming into the stroma, and these may be of stromal rather than follicular origin [23].

Usually the granulosa cells are inconspicuous and may have involuted. However, occasionally the follicles are preserved and expanded and hyperplasia of partially luteinized granulosa cells may be seen [36].

Differential Diagnosis: When multiple, inspection of the ovaries at operation may suggest the presence of nodules of metastatic tumor, and the differential diagnosis in this situation includes metastatic carcinoma and metastatic melanoma. For unilateral pregnancy luteomas, various primary ovarian tumors are in the differential, but the clinical history of pregnancy will usually point to the correct diagnosis. Although thecomas may be luteinized in pregnancy, the basic spindle cell shape is still evident. Sclerosing stromal tumors have a lobular pattern with bands of collagen. Abundant pericellular reticulin is seen in both thecomas and sclerosing stromal tumors. Steroid cell tumors are usually unilateral and usually arise at the ovarian hilus, reticulin surrounds single cells or small groups, and mitotic activity is usually less prominent than in stromal luteomas of pregnancy. Whereas stromal luteomas have relatively little stainable intracytoplasmic lipid, steroid cell tumors show abundant lipid content. Reinke crystals may be seen in Leydig cell tumors. Granulosa cell tumors are rare in pregnancy but when present are often of the juvenile type which may display prominent luteinization and therefore may resemble pregnancy luteoma.

Hyperreactio Luteinalis

In hyperreactio luteinalis, numerous follicular cysts are the manifestation rather than theca cell expansion. In common with nodular theca-lutein hyperplasia, hyperreactio luteinalis (multiple theca-lutein cysts) is also associated with increased βhCG, but, in contrast with pregnancy luteomas, hyperreactio luteinalis is also associated with pathological βhCG elevations. It is associated with hyperstimulation of the ovaries such as in pregnancy [37, 38], especially multiple

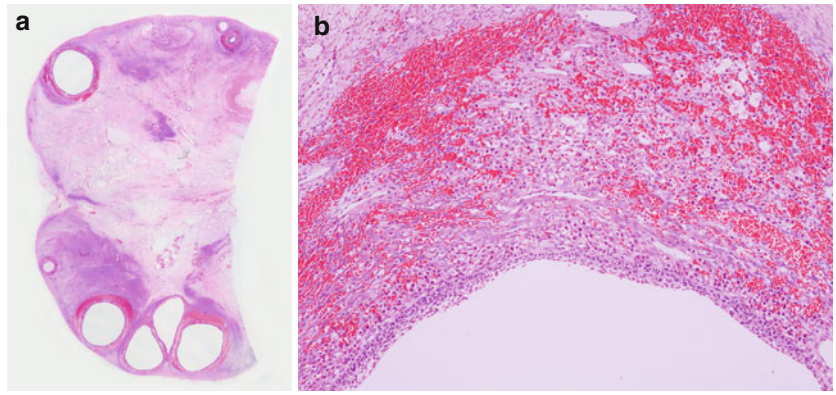

Fig. 2.8 (**a**) Hyperreactio luteinalis. Low-power view showing multiple cystic follicles in an ovary removed at 22 weeks gestational age. (**b**) Hyperreactio luteinalis. Follicle cyst lined by luteinized cells. The adjacent stroma also contains luteinized cells

pregnancies [39], gestational trophoblastic neoplasia, and treatment for ovulation induction [40]. The classical association is with hydatidiform mole and choriocarcinoma, and hyperreactio luteinalis occurs in 25 % of cases. The presentation in the majority of cases is in the third trimester or peripartum period.

Clinical Features: The condition may present as a clinically detectable ovarian mass seen on ultrasound or at caesarean section [40, 41]. Pelvic pain, torsion, and cyst rupture are rare, as is virilization of the mother [42]. The cysts may persist postpartum despite reduced βhCG levels; this may be explained by high LH and FSH levels postnatally if lactation is not established. Regression will occur within a few weeks after parturition but may take up to 6 months [43].

Histopathological Findings: Macroscopically there is bilateral expansion of the ovaries which may reach up to 15 cm diameter. The cut section shows numerous small cysts 1–4 cm diameter that contain yellowish watery fluid or blood. The surrounding stroma is edematous. Microscopically both granulosa cells and theca interna cells may be luteinized, and the follicles may appear hyperplastic (Fig. 2.8).

Differential Diagnosis: Clinical recognition is important to avoid unnecessary surgery during pregnancy with the erroneous clinical impression of malignancy; recognition of this condition will allow conservative management. Solitary luteinized follicular cyst of pregnancy may enter the histopathological differential, but these are solitary and large and granulosa and theca cell layers are not distinct.

Solitary Luteinized Follicular Cyst of Pregnancy and the Puerperium

This is a large distinctive follicular cyst that arises in pregnancy or the puerperium [44, 45]. They usually present in the 3rd and 4th month of pregnancy with a clinically or ultrasound-detected mass, as an incidental finding at caesarean section or in the puerperium. They regress spontaneously after delivery [46]. Hormonal dysregulation is not a feature [47]. The pathogenesis is not known, but stimulation with βhCG is likely to be important.

Macroscopically there is a unilateral unilocular cyst containing watery fluid. The mean size has been reported in one series as 25 cm [47]. Microscopically the cyst is lined by a single layer or multiple layers of large luteinized cells with abundant eosinophilic cytoplasm and large hyperchromatic and pleomorphic nuclei. Mitoses are not seen.

The main differential diagnosis is with a cystic ovarian tumor, particularly in view of the nuclear pleomorphism and hyperchromasia. Awareness of the entity in pregnancy is a major factor in reaching the correct diagnosis.

Ectopic Decidua

The finding of ectopic decidua on the ovarian surface and in the ovarian cortex is common in normal intrauterine pregnancy. When prominent, it forms small nodules on the ovarian surface which, if noted at caesarean section, may cause concern regarding carcinomatosis [48]. The formation of ectopic decidua on the ovarian surface

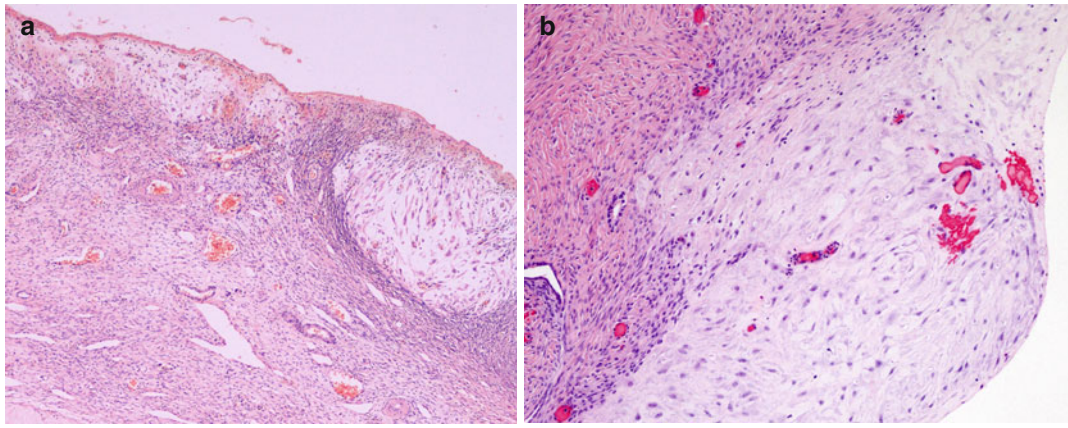

Fig. 2.9 (**a**) Ectopic decidua. Low-power view of decidualization of ovarian subserosa. (**b**) Ectopic decidua. This example was associated with an ovarian adenofibroma

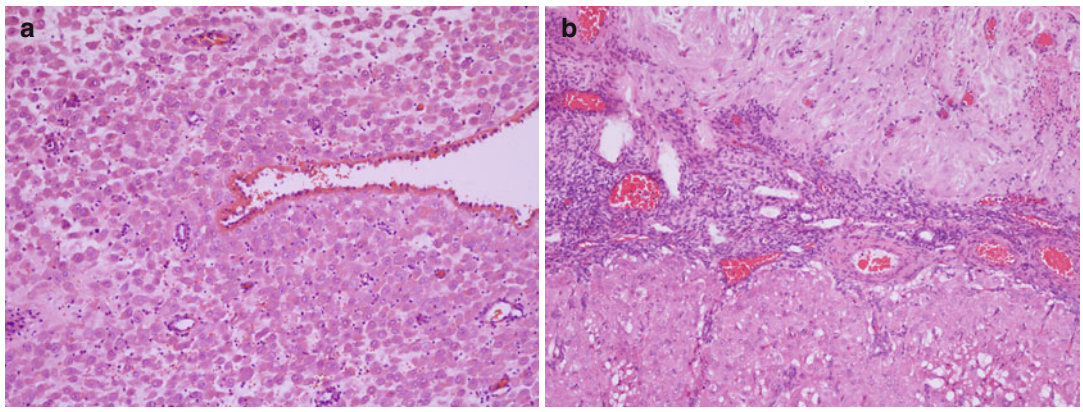

Fig. 2.10 (**a**) Decidualized endometriosis. The ovary was removed at the time of caesarean section performed for placenta accreta. There is prominent stromal decidualization of the endometriotic foci. (**b**) Decidualized endometriosis. Decidualization of ovarian stroma (*top*) in the same case as (a). A corpus luteum is seen in the lower part of the photomicrograph

(Fig. 2.9) and in the cortex is a physiological process arising from the coelomic mesenchyme as a result of progesterone stimulation in pregnancy. It is seen in most pregnancies in the 3rd trimester, but is less common in earlier pregnancy. Decidual change is also seen in endometriotic foci within the ovary in pregnancy (Fig. 2.10).

Macroscopically the ovarian surface shows tan or red spots up to 5 mm diameter. Microscopically the component cells are similar to those seen in decidualized endometrium; the decidualized stromal cells have distinct margins and are polygonal in shape with eosinophilic cytoplasm and a central pale nucleus with prominent nucleolus (Figs. 2.9 and 2.10). As in uterine decidua, there is a prominent leukocyte component, including uterine natural killer cells and macrophages [49]. The main differential is with smooth muscle metaplasia, but the clinical context should ensure a correct diagnosis, which can if necessary be confirmed by immunostaining of decidualized endometrial stromal cells for CD10.

Ovarian Ectopic Pregnancy

Ectopic pregnancy within the ovary is a rare event, occurring only around 1 in 10,000 pregnancies and accounting for 0.94–3.6 % of ectopic gestations [50–54] and up to 6 % of pregnancies

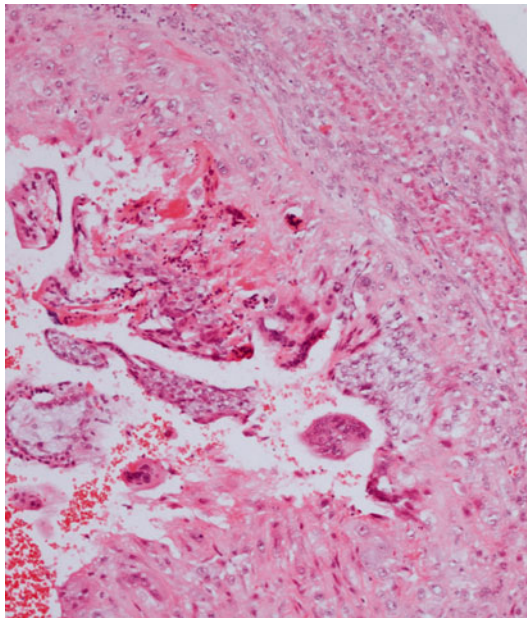

Fig. 2.11 Ovarian ectopic pregnancy. Immature chorionic villi and implantation site are seen with luteinized cells in adjacent stroma

conceived following assisted conception [55]. A definite diagnosis of an ovarian ectopic pregnancy requires exclusion of an ectopic tubal pregnancy with secondary involvement of the ovary. Hence, for a diagnosis of ectopic ovarian pregnancy, the tube must be intact and separate from the ovary; definite ovarian tissue should be present in juxtaposition to trophoblast (Fig. 2.11), and the fetal sac should occupy the normal position of the ovary, with removal resulting in the serum βhCG returning to normal [3]. The relationship between ovarian ectopic pregnancy and use of an IUCD or the presence of pelvic inflammatory disease is not clear with some reporting an association and others not [56]. It is thought that an ovum is likely to undergo fertilization in the fimbria or on the ovarian surface and then implants within ovarian parenchyma.

Inflammatory Conditions

The ovary can be involved in inflammatory processes due to a range of infectious agents, as well as other inflammatory conditions unrelated to infection. The histopathological features in oophoritis due to a range of infections have recently been summarized [57].

Ovarian Inflammation Associated with Infection

Bacterial Infection and Pelvic Inflammatory Disease

Oophoritis due to bacterial infection is usually seen in the context of pelvic inflammatory disease and also involves the fallopian tubes. Recurrent infectious episodes may lead to the formation of a tubo-ovarian abscess, and the inflammatory exudate often leads to the development of adhesions with adjacent structures. Isolated ovarian abscess not associated with salpingitis is uncommon and is usually associated with a predisposing factor such as a recent gynecological operation, childbirth, or complication of intrauterine contraceptive device (IUCD) use. Occasional infections of the ovary arise as a result of spread from intestinal infection such as appendicitis or diverticulitis or a postoperative infection. Whereas oophoritis and abscess associated with PID tend to be insidious, that occurring in isolation may present acutely and require surgical intervention with peritonitis and pelvic abscess as possible sequelae [57].

The presenting features in infectious oophoritis are usually those of pelvic inflammatory disease with pain, fever, and vaginal discharge and sometimes urinary symptoms. In advanced disease, there may be development of a tubo-ovarian mass, and adhesions are a common consequence. The rupture of an abscess occasionally leads to secondary peritonitis or fistula formation, and occasionally healing of an abscess leads to the formation of a cystic structure that can be confused with a simple cyst. Subclinical infections are common, and a history of PID is present in only 50 % cases with tubo-ovarian abscess [58].

Macroscopically there may be a tubo-ovarian mass and abscess formation, often in continuity with the fallopian tube. Milder cases may be limited to showing peri-oophoritis. Rarely in a recurrent infection, a chronic abscess forms

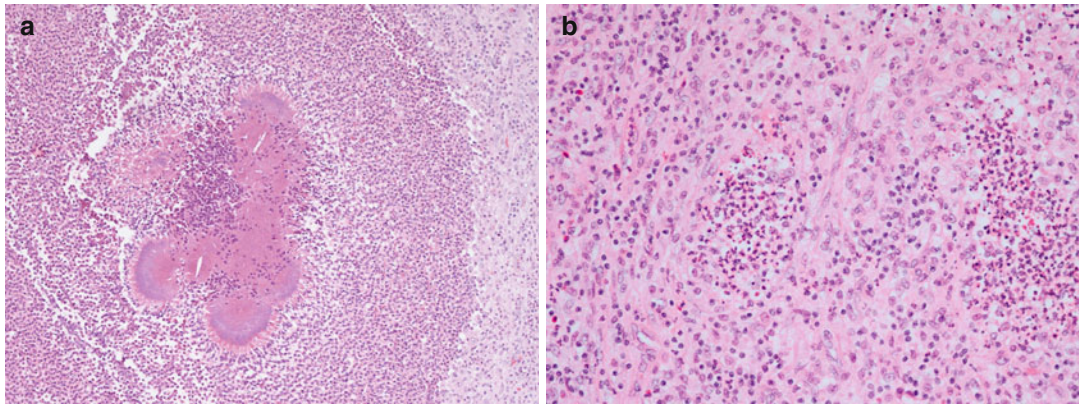

Fig. 2.12 (**a**) Actinomycosis. A colony of actinomyces ("sulfur granule") surrounded by an acute inflammatory cell infiltrate in a case of actinomycosis associated with a tubo-ovarian mass. (**b**) Actinomycosis. Detail of inflammatory infiltrate consisting of neutrophil polymorphs and foamy macrophages admixed with lymphocytes and plasma cells

with the development of a yellow solid mass, termed xanthogranulomatous oophoritis [59]. Microscopically there are the usual features of acute inflammation with neutrophil polymorphs, edema, and vascular dilatation. Abscess formation is usually in continuity with the fallopian tube and may result in permanent loss of ovarian parenchyma. Peri-oophoritis shows neutrophils and fibrin with formation of granulation tissue and sometimes prominent mesothelial proliferation. Rarely an abscess may evolve into xanthogranulomatous oophoritis with accumulation of macrophages, multinucleated giant cells, plasma cells, neutrophil polymorphs, and fibrosis [60].

Actinomycosis

Actinomycosis is uncommon, and most cases occur after prolonged use of an IUCD [61–64]. Despite this association, most cases of IUCD-associated PID are not due to actinomycosis [63]. The organism may be identified in cervical smears of IUCD users, and a substantial proportion of those with positive smears will develop clinical actinomycosis infection and tubo-ovarian abscess [65]. Rarely actinomycosis is not associated with IUCD use [66].

Macroscopically there is a large tubo-ovarian mass which can be unilateral or bilateral and in some cases may mimic pelvic malignancy [67]. Sectioning shows multiple abscesses involving the fallopian tube and ovary and separated by dense scar tissue. The characteristic sulfur granules may rarely be visible macroscopically.

Microscopically there is a nonspecific inflammatory response with neutrophils, foamy histiocytes, lymphocytes, and plasma cells (Fig. 2.12). Bacterial colonies are identified as branching filaments with a basophilic central zone with more peripheral palisading zones appearing eosinophilic (Fig. 2.12). The organisms are Gram positive and also stain with silver stains.

Tuberculosis

The ovaries are only involved in 10 % cases of tuberculosis of the female genital tract, whereas the fallopian tubes are involved in almost all cases. Although most genital tuberculosis is thought to be blood-borne, fewer than 50 % cases have a history of tuberculosis or an abnormal chest x-ray [57]. Ovarian tuberculosis usually results from direct spread from the fallopian tubes, which are almost always involved. There is obliteration of the normal anatomical relationship between the fallopian tube and ovary [23]. Less commonly spread may occur from the gastrointestinal or urinary tract via lymphatics. The intraoperative appearance may mimic ovarian cancer [68, 69]. Microscopically the histological features are similar to those observed elsewhere in the body, although there is rarely caseation in the ovary and the granulomatous inflammation is often confined to the ovarian cortex. Culture or

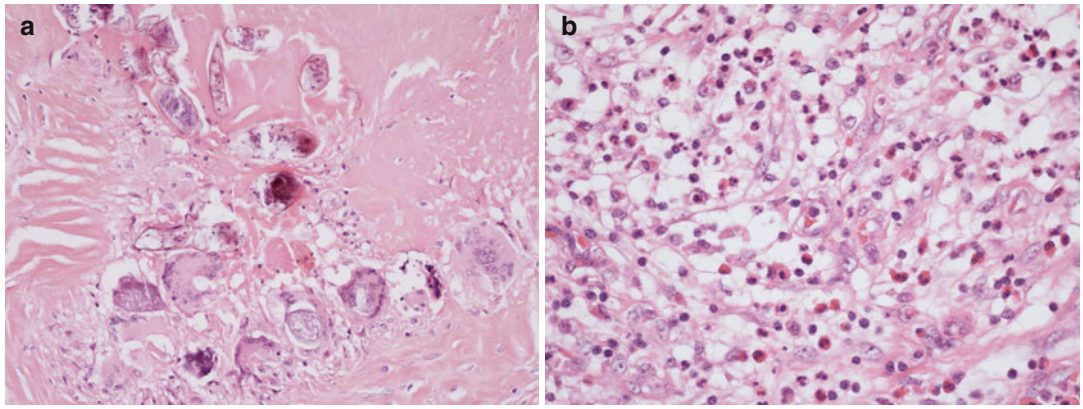

Fig. 2.13 (**a**) Tubo-ovarian schistosomiasis. Degenerate and calcified schistosoma ova surrounded by dense scarring. (**b**) Tubo-ovarian schistosomiasis. Detail of eosinophil-rich inflammatory cell infiltrate

polymerase chain reaction may be helpful to confirm the diagnosis.

Viral Infections

Mumps

Oophoritis may occur in mumps infection, although it is a less common manifestation of this infection than mumps orchitis in males, with estimates of around 5 % of females suffering mumps infection [29, 57]. The histopathological features of mumps oophoritis have not been described.

Cytomegalovirus

Cytomegalovirus can also affect the female genital organs, usually secondary to viremia. Oophoritis is rare and has been reported only in immunocompromised patients [70, 71]. On microscopy infected stromal and endothelial cells are readily identified by their enlargement and pleomorphism and by the characteristic intranuclear inclusions. Immunohistochemistry may help to confirm the diagnosis [72].

Parasitic Infection

Schistosomiasis

Parasitic infections of the ovary are rare, but ovarian schistosomiasis is commonly seen in endemic areas [73]. The worms can gain access to the genital tract via venous anastomoses such as between mesenteric and ovarian veins. Ovarian schistosomiasis may be an incidental finding with normal-sized ovaries, but patients may have pelvic pain or pelvic mass and occasionally irregular menstrual cycle or infertility. The intraoperative findings may be of an enlarged ovary and fallopian tube with abscesses, adhesions, and necrosis and can simulate a malignant tumor. Microscopically there are schistosoma ova surrounded by a granulomatous infiltrate, which usually contain eosinophils as well as lymphocytes, plasma cells, and foreign body giant cells (Fig. 2.13). The inflammatory response may be predominantly eosinophilic. The peripheral zones show fibrosis that increases in amount as the lesions progress and may replace them. Dead ova often undergo calcification. Schistosoma ova can be identified and differentiated by their spines; *Schistosoma haematobium* has a terminal spine, while *S. mansoni* has a lateral spine as does *S. japonicum*, although the lateral spine is less prominent. *S. haematobium* preferentially involves the lower genital tract, whereas *S. mansoni* has a predilection for fallopian tubes and ovaries [57].

Enterobiasis

The involvement of the ovarian surface or rarely parenchyma by *Enterobius vermicularis* is usually an incidental finding [74, 75]. On reaching the peritoneal cavity, the worms die and the ova are released and stimulate an inflammatory response. The granulomas, which contain eosinophils, are localized in the pelvis and may involve the ovarian serosal surface or parenchyma [76]. *Enterobius* ova are smaller than those of schistosoma and lack spines.

Echinococcus

This is an infestation by the cestode *Echinococcus granulosus*, and there are several reports in the literature documenting rare cases of ovarian involvement [77–80]. Ovarian involvement may represent primary or secondary infection and presents clinically or on ultrasound examination as ovarian cysts. The cystic mass may mimic an ovarian cystic neoplasm ovarian cystic neoplasm or endometriosis [80]. The histopathological features are those of a typical hydatid cyst with reports of cysts measuring up to 16 cm diameter. The cysts have a characteristic lining and regress after death of the parasite; cyst wall calcification after regression may be a useful radiological sign.

Fungal Infection

Fungal infection of the ovary is extremely rare even in the presence of disseminated disease. Ovarian involvement by blastomycosis [81, 82] and coccidioidomycosis [83] has been reported, and a case of aspergillus has been reported in an IUCD user [84].

Noninfectious Inflammatory Conditions of the Ovary

The ovary can be affected in a range of noninfectious inflammatory diseases associated with granulomatous inflammation. Sarcoidosis has been reported to rarely involve the female genital tract and may affect the uterus and adnexae [85]. Granulomas are distributed throughout the ovary and are usually an incidental finding. The ovary is also rarely involved by Crohn's disease [86, 87], usually by direct extension of the inflammation from the affected bowel.

Giant cell arteritis may affect smaller arteries and arterioles, and the female genital tract can be affected [88]. Female genital tract involvement in polyarteritis nodosa is most common in the cervix but occasionally affects adnexae [89]. Rarely the ovary shows necrotizing arteritis with no underlying cause [90]. These are considered to be cases of isolated visceral necrotizing vasculitis; systemic disease should be sought, but follow-up rarely uncovers any underlying explanation [90, 91].

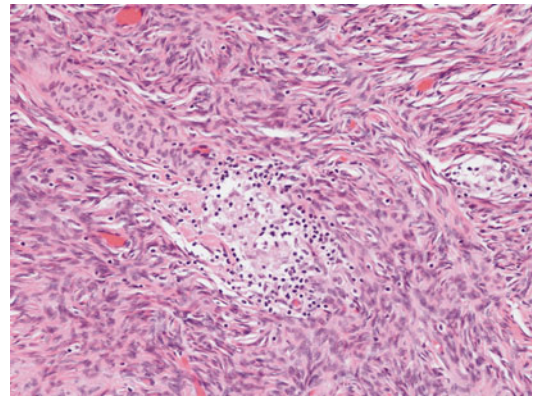

Fig. 2.14 Cortical granulomas. Well-circumscribed granulomas consisting of epithelioid cells and lymphocytes in the ovarian cortex of a postmenopausal woman

Cortical granulomas are seen in 10–15 % of postmenopausal ovaries, usually in association with stromal hyperplasia and hyperthecosis, and it is possible that they represent transitional stages in the involution. The granulomas are circumscribed and show epithelioid cells and lymphocytes, and sometimes multinucleate giant cells and fat crystals (Fig. 2.14). Older lesions are fibrotic and hyalinized.

A granulomatous response to foreign material such as suture material, lipid contrast material, and keratin may be seen in the ovary and can mimic a malignant tumor [92]. Foreign body inflammation in response to bowel contents has also been reported in colo-ovarian fistula [93].

Isolated noninfectious granulomas typically occur in premenopausal women without evidence of systemic granulomatous disease or granulomatous genital tract infection. In most patients, there is a history of surgery involving the affected ovary months or years before, suggesting that the lesions represent a reaction to trauma or tissue necrosis. The lesions resemble the necrobiotic rheumatoid-like granulomas that are found in the cervix after loop excision of the transformation zone [86].

Autoimmune Oophoritis

Premature ovarian failure can be divided into four broad etiological categories: genetic, autoimmune, iatrogenic, and environmental [94].

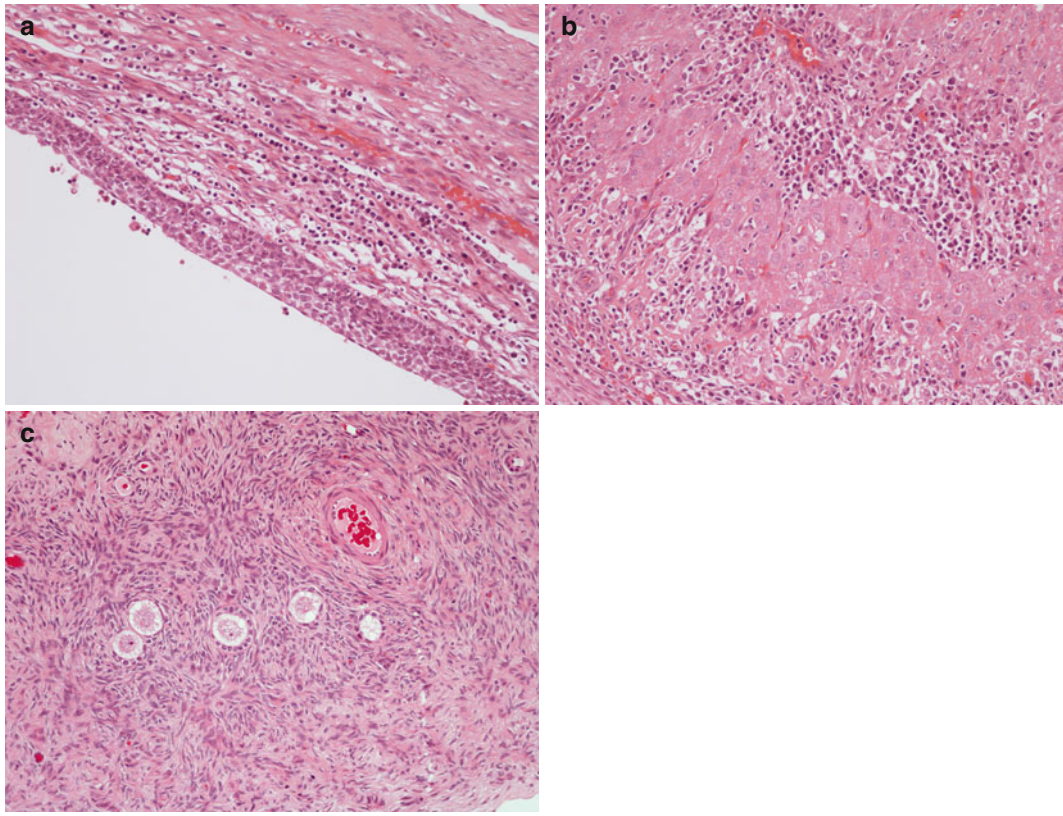

Fig. 2.15 (**a**) Autoimmune oophoritis. Cystic follicle showing lymphocyte infiltrate in the theca interna. (**b**) Autoimmune oophoritis. Intense lymphocyte infiltrate in a fresh corpus luteum. (**c**) Autoimmune oophoritis. Sparing of primordial follicles

There is a strong association between premature ovarian failure and a range of autoimmune disorders [95–98]. A presumed mechanism is the presence of autoantibodies directed against steroid-producing cells, and these can be detected in various autoimmune diseases, especially thyroid and adrenal disease [99]. However, some patients with the clinicopathological features of autoimmune oophoritis do not have anti-ovarian antibodies [100]. Studies of mouse models of the disease have suggested that there is an immune defect, including a reduction in natural killer cell activity, allowing the development of organ-specific autoimmunity and that there is an ovarian target under attack. However, as yet the specific immune defect or the ovarian target is not fully understood [101, 102].

Autoimmune oophoritis usually presents as hypergonadotropic ovarian failure with primary or secondary amenorrhea and premature menopause and infertility [103]. Laboratory studies may confirm the presence of anti-ovarian antibodies, and some patients will have a history of other organ-specific autoimmune disease.

Macroscopically the ovaries are normal in size or enlarged by cystic follicles. On microscopic examination, there is an inflammatory cell infiltrate directed at follicular cells. The infiltrate usually consists of lymphocytes and plasma cells, but sometimes there is an eosinophilic infiltrate or a granulomatous inflammatory response [103–105]. The inflammatory infiltrate is closely related to the theca interna of developing follicles, and the more advanced the follicular development, the denser the inflammatory infiltrate. The granulosa cells are only sparsely infiltrated by inflammatory cells, and primordial follicles are spared (Fig. 2.15). The intervening stroma is normal.

The differential diagnosis includes other causes of oophoritis, but the perifollicular distribution of the inflammation is characteristic.

Endometriosis

Introduction

Endometriosis is defined as the presence of functional endometrial tissue located outside the endometrial cavity. It mainly affects women in their reproductive years, although postmenopausal women may be affected, usually associated with exogenous or endogenous hormone production. The incidence is uncertain, but estimates are that around 10 % of women of reproductive age are affected [106, 107], although this may be an underestimate since the diagnosis may be made only several years after onset of symptoms [108].

Factors that have been associated with endometriosis are Caucasian or Asian race, although more recent studies have cast doubt on the importance of racial factors [109]. Other factors suggested to be associated with an increased risk of endometriosis are lower BMI at age 18 years, long cycle length, reduced parity, and use of IUD, whereas oral contraceptives were protective [110]. There is some indication that genetic factors play a role since the incidence is higher in monozygotic than dizygotic twins [111].

Clinical features vary and include infertility; lower abdominal, pelvic, and back pain; dyspareunia; and dysmenorrhea, but only 5 % of women have all four major symptoms. Many women are asymptomatic, and in one study asymptomatic endometriosis was detected in 40 % of women undergoing laparoscopic tubal ligation [112]. The clinical features correlate poorly with disease extent; women with minimal disease may have severe pain, and those with extensive disease may have relatively few symptoms [113]. Rare complications are formation of a pelvic mass, ascites, hemoperitoneum, and infection or rupture of an endometriotic cyst.

Various theories have been put forward to explain the pathogenesis and pathophysiology of endometriosis [reviewed in [114]]. These include coelomic metaplasia, the induction theory, mullerianosis, and benign metastasis. A more recent proposal suggests that extrauterine stem or progenitor cells that originate from the bone marrow may differentiate into endometrial tissue [115]; candidates are mesenchymal progenitor cells and endothelial progenitors. The most popular theory of retrograde menstruation proposes that the endometrium is shed into the peritoneal cavity at menstruation, implants, and grows into endometriotic foci. There is strong evidence for retrograde menstruation: menstrual blood is found within the peritoneum in over 90 % of women at the time of menstruation; the anatomical distribution of endometriotic lesions is in keeping with retrograde menstruation; and outflow obstruction in vivo due to congenital abnormalities or induced experimentally predisposes to endometriosis. Retrograde menstruation cannot, however, explain the development of endometriosis since this occurs as a normal feature in menstruating women. Endometrial fragments must differ in their ability to survive and grow in women who develop endometriosis and those who do not, and various active areas of research are focusing on this area.

Ovarian Endometriosis

The frequency of sites affected by endometriosis depends on whether diagnosis is based on clinical or histological findings [116]. Clinically the two most frequent sites are the uterosacral ligaments and the ovaries, with other sites in the pelvis accounting for the majority of others, including the pouch of Douglas, pelvic peritoneum, uterine serosa, and fallopian tubes [117]. Endometriosis is often encountered incidentally in bilateral salpingo-oophorectomy samples removed for unrelated reasons, and ovarian endometriosis is the most common site where endometriosis is seen by histopathologists [118]. Extrapelvic disease is seen in 5–12 % of patients clinically, with the intestines the most commonly involved site.

Macroscopic Findings: Ovarian endometriosis has several different patterns which may coexist. Endometriotic lesions on the ovarian surface may be yellow/red lesions in their early stages, bluish/black due to bleeding, brownish yellow due to the presence of hemosiderin, or whitish due to fibrosis and scarring in old lesions. Endometriotic lesions increase in size as they become older, and there may be associated dense

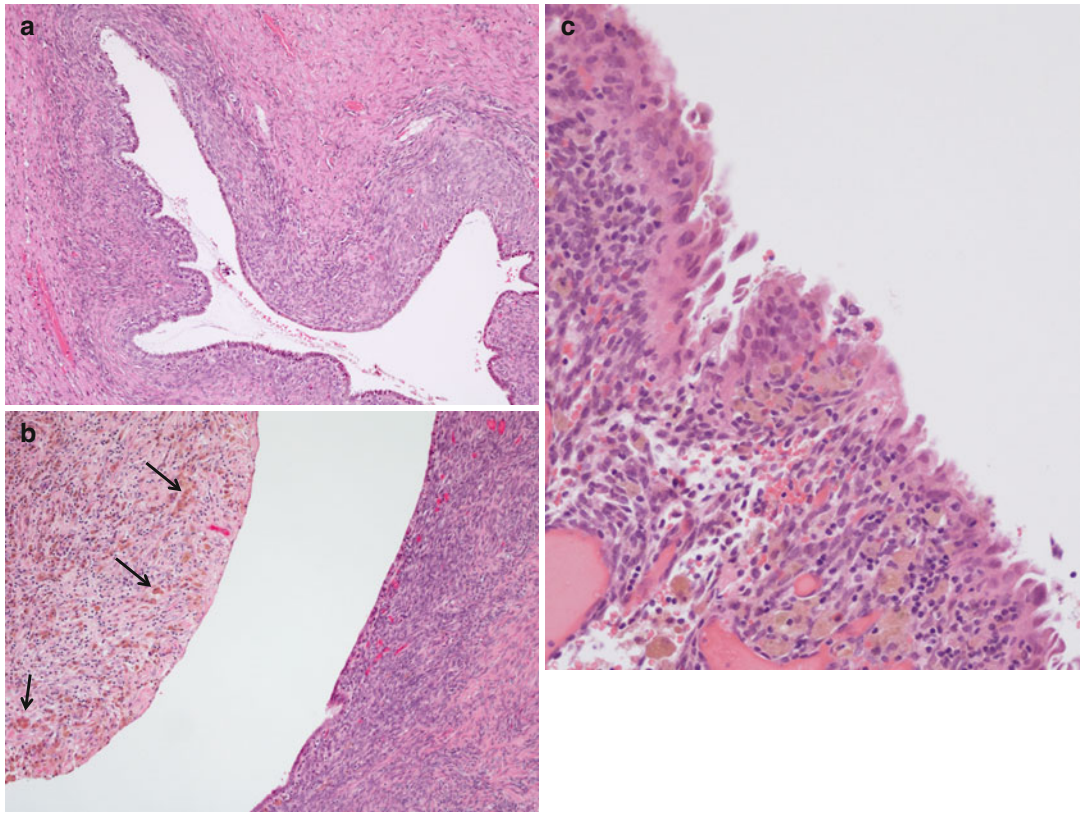

Fig. 2.16 (**a**) Ovarian endometriosis. Typical focus of endometriosis showing easily recognizable endometrial glands and stroma. The stroma was strongly immunoreactive for CD10. (**b**) Ovarian endometriosis. Two sides of an endometriotic cyst. The right side is easily recognizable as endometriosis, whereas the left side shows loss of the epithelium and substantial replacement of the stroma by hemosiderin-laden macrophages (*arrows*). (**c**) Ovarian endometriosis. Reactive atypia in ovarian endometrioma

fibrous adhesions. Cortical endometriosis may have a similar macroscopic appearance, but small lesions may not be visible macroscopically.

The typical endometriotic cyst or endometrioma is a common cause of an ovarian mass in the fourth and fifth decades [119]. The size ranges from a few mm up to >15 cm diameter. The contents are watery or hemorrhagic, and inspissated old blood accounts for the term "chocolate cyst." The cyst wall is of varying thickness and is usually fibrotic [120] with a smooth or shaggy brown lining. Patches of discoloration may be present due to hemorrhage or hemosiderin deposition, and rarely the cyst lining has a cobblestone pattern associated with stromal decidualization within the endometriotic lesions. Endometriotic cysts are associated with hemorrhage, inflammation, and adhesions, which may result ultimately in the formation of a complex ovarian mass which requires careful sampling to exclude malignancy. Because of their complex appearance, endometriotic cysts may be mistaken for malignancy on ultrasound examination, an impression which may be reinforced by a moderately raised CA125 level, a common finding in endometriosis. Since endometriosis has an association with malignancy, any intraluminal polypoid lesions or mural nodules in endometriosis require careful sampling. Endometriotic cysts are often bilateral, and microscopic endometriosis is often detected in the contralateral ovary.

Microscopic Findings: Microscopically, the classical hallmarks of endometriosis are the presence of endometrial glands and stroma (Fig. 2.16a). There is usually evidence of previous hemorrhage with hemosiderin deposition in stromal macrophages. The endometrial glands may be inactive or have features of proliferative or secretory activity. The stromal component

resembles endometrial stroma with a typical network of arterioles. These typical and easily recognizable features, however, represent a minority of cases, and endometriotic cysts often present as a fibrous-walled cyst with a rather indistinct lining. The stroma may be inconspicuous and form only a thin cuff. Sometimes the typical endometrial stroma may be obscured by macrophages, or in long-standing lesions the stromal cells may appear more fibrotic (Fig. 2.16b). The presence of hemosiderin-laden macrophages may be a helpful feature, along with the presence of arterioles similar to those seen in the endometrium. The stroma may also undergo metaplastic changes, particularly smooth muscle metaplasia. The lining epithelium may be attenuated and nondescript, and sometimes a definite diagnosis cannot be made. Metaplastic changes may also occur in the epithelium, including tubal, hobnail, and rarely mucinous and squamous metaplasia.

Epithelial cells may show atypia characterized by nuclear enlargement and hyperchromasia with abundant eosinophilic cytoplasm (Fig. 2.16c). These changes are regarded as reactive in most cases with no associated increased risk of malignancy [121], although occasionally the changes merge with neoplasia within a cyst, suggesting premalignant potential in rare cases [122]. In the presence of pregnancy or after high-dose progesterone treatment, foci of endometriosis may show prominent decidualization which may lead to confusion with a corpus luteum or xanthomatous change. Unusual histological features are the presence of a xanthomatous nodule lacking typical endometrial glands, which are considered to represent burnt-out endometriotic foci. Stromal endometriosis is endometriosis that lacks the epithelial element and has been reported at several sites [123] and may occur within the ovarian cortex.

The diagnosis of endometriosis may be difficult. The endometrial lining epithelium may be attenuated and cuboidal in appearance and if atrophic may resemble a simple cystadenoma lining. Endometrial stroma may be minimal, but even a minute amount can identify the cyst as endometriotic. Immunostaining for CD10 may help highlight endometrial stroma in subtle lesions, although it is important to recognize that this antibody is not specific for endometrial stroma. In rare cases, the endometrial lining of an endometriotic cyst is completely lost, and the stroma has been effaced by repeated hemorrhage. The residual appearance may be of hemosiderin-laden macrophages within a fibrous wall, and in this situation the diagnosis cannot be made with certainty.

Differential Diagnosis: The differential diagnosis of ovarian endometriosis includes ovarian inclusion cysts and glands, but these lack the typical surrounding endometrial stroma, although this can be subtle. Inclusion cysts are usually lined by cuboidal or ciliated cells rather than endometrial cells and may be associated with psammoma bodies. The presence of macrophages and hemosiderin deposition suggests endometriosis, and CD10 immunoreactivity may help to clarify the diagnosis of endometriosis. Some apparent cortical inclusion cysts may show ill-defined weaker reactivity for CD10, and the diagnosis may be impossible to determine with certainty [23]. Rete ovarii may superficially resemble endometriosis, but the glands have a characteristic ramifying pattern, are lined by cuboidal or columnar cells and are surrounded by condensed ovarian stroma rather than endometrial stroma.

Other rare differential diagnoses include extrauterine stromal sarcoma versus stromal endometriosis, but the latter rarely forms masses and lacks stromal mitotic activity and vascular invasion. Corpus luteum cysts may be confused with endometriosis both macroscopically and microscopically since both may show a ragged internal surface and hemorrhage, but endometrial glands and stroma are absent, and corpus luteum cysts usually contain aggregates of luteinized theca cells.

Development of Malignant Change in Endometriosis

Ovarian endometriosis may undergo similar changes to those seen in the endometrium. Formation of endometrial polyps gives rise to polypoid endometriosis, and endometriotic lesions at any site may undergo hyperplasia ranging from simple hyperplasia to atypical complex hyperplasia.

The incidence of malignancy in endometriosis is uncertain. Florid endometriosis may form tumorlike masses which can be confused with a malignant tumor clinically and macroscopically. A malignant tumor arising in endometriosis may lead to destruction of the associated endometriosis or extensive sampling may be necessary to detect the endometriosis from which the tumor has arisen. The incidence of malignant tumors arising in ovarian endometriosis is cited as 0.3–0.8 % by some authors, but others have detected higher levels of 1.1–3 % [118, 124]. The variation in incidence is likely to reflect differences in sampling and the precise criteria used to determine whether a malignant tumor has arisen in endometriosis. Endometrioid carcinoma accounts for the majority of cases (70 %) and clear cell carcinoma for 4 %. However, although endometrioid carcinoma is the most common tumor type in endometriosis, the association with clear cell carcinoma is stronger with 25–50 % cases being associated with endometriosis. Other rarer tumors that may arise are borderline endometrioid adenofibromas, stromal sarcoma, squamous cell carcinoma, and carcinosarcoma.

Miscellaneous Lesions

Surface Epithelial Inclusion Cyst

The normal ovarian surface epithelium is a single layer of modified mesothelial cells. Invagination of the ovarian surface epithelium results in glandular structures within the ovarian cortex, and these may develop into inclusion cysts. Ovarian inclusion cysts are thought to arise as part of the healing reaction after follicular rupture, but they could also result from entrapment in surface adhesions. The frequency of inclusion cysts increases with age, and they are common in late reproductive age and postmenopausal women. They are asymptomatic and detected as an incidental finding.

Macroscopically inclusion cysts are often multiple and scattered in the cortex, although rarely may involve the medulla. By convention they are <1 cm diameter, larger cysts being considered to be cystadenomas. Microscopically

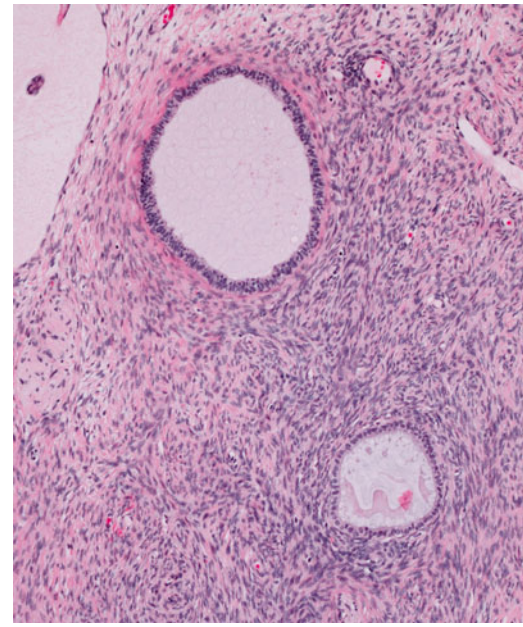

Fig. 2.17 Cortical inclusion cyst. Cyst lined by cuboidal epithelium within the cortex of an ovary from a postmenopausal woman

inclusion cysts are lined by nonspecific flattened or cuboidal epithelium (Fig. 2.17) or benign tubal-type ciliated epithelium [125]. Psammoma bodies may be seen in the cysts or in adjacent stroma. Less commonly the cysts are lined by epithelium of other Mullerian type such as mucinous endocervical-type or endometrial-type epithelium. The differential diagnosis includes atrophic endometriosis.

Inclusion cysts are found in the majority of ovaries from postmenopausal women. Their importance arises from the role of inclusion cysts in the development of surface epithelial neoplasms. This conclusion was based on the detection of increased numbers of surface inclusion cysts in the contralateral ovary of women with ovarian cancer compared with controls. In addition, occasionally inclusion cysts show dysplastic changes and immunoreactivity for a range of epithelial ovarian tumor markers. More recent evidence has, however, suggested alternative origins, and the relative contributions to the pathogenesis of epithelial ovarian cancer between ovarian cortical inclusion cysts, fallopian tube, endometrium, and peritoneum remain unclear [125–127].

Rete Cysts

The rete ovarii is the ovarian analogue of the rete testis and is present in the hilus. There is a network of branching tubule lines by the epithelium which is flattened, cuboidal, or columnar, and there is a stromal cuff of ovarian type stroma. Occasionally the rete may develop into a hilar cyst or, if >1 cm diameter, a cystadenoma. These are usually unilocular and have been reported to have a mean diameter of 8.7 cm (range 1–24 cm) [128]. The lining epithelium is a single layer of non-ciliated epithelium. Clues to the origin are the hilar location, presence of muscle, hyperplastic hilus cells within the wall, and an irregular inner lining.

Simple or Indeterminate Cysts

The precise nature of some ovarian cysts may be impossible to determine with certainty. The lining epithelium may be attenuated and nonspecific resembling epithelial or mesothelial cells, or the epithelium may be lost by trauma or desiccation. The cyst contents are usually nonspecific and watery. Additional sampling may allow more definitive identification of a cyst as serous or endometrioid, and other features such as hemosiderin-laden macrophages within the wall may suggest a specific diagnosis, such as endometriosis or hemorrhagic corpus luteum cyst. A rare additional possible cause of a nonspecific cyst is a cystic struma ovarii with inconspicuous thyroid follicles within the wall, a diagnosis that may become clear with further sampling. In many cases, however, definitive identification is not possible, and the final diagnosis has to be made of an indeterminate or simple cyst with no evidence of malignancy.

Mesothelial Proliferations

Mesothelial proliferation on the ovarian surface may arise as a response to pelvic inflammation but can also be seen in response to ovarian tumors and endometriosis [129–131]. In florid cases, there may be complex papillary or glandular mesothelial cell proliferations which may simulate neoplastic epithelial cells. Distinction can be made with immunohistochemistry for mesothelial markers such as calretinin, thrombomodulin, and D2/40 and epithelial markers such as BerEp4 and epithelial membrane antigen (EMA).

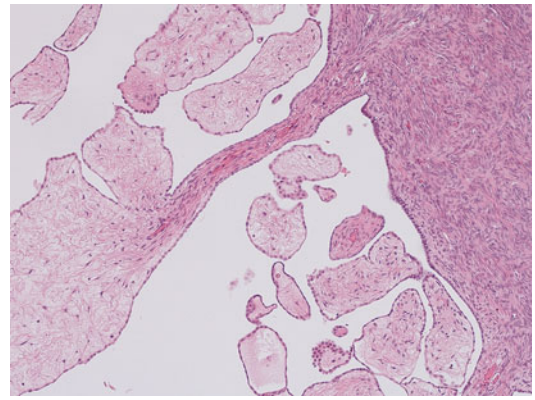

Fig. 2.18 Surface stromal proliferation. Incidental finding in an ovary removed at the time of hysterectomy. A size limit of 10 mm arbitrarily separates these from surface serous papillomas

Surface Stromal Proliferations

Nodular or papillary surface stromal projections are a common incidental finding on microscopic examination of the ovaries of postmenopausal and late reproductive age women. These are composed of ovarian stroma with varying hyalinization and a covering of a single layer of epithelial cells (Fig. 2.18). A size limit of 10 mm arbitrarily separates these proliferations, which are usually multiple, from surface serous papillomas.

Ovarian Remnant Syndrome

Ovarian remnant syndrome is defined as the presence of symptomatic ovarian tissue after bilateral salpingo-oophorectomy [132]. It is believed to result from unintentionally leaving ovarian tissue behind after a difficult operative procedure, often associated with adhesions such as in pelvic inflammatory disease or endometriosis [133]. Often the patient has had multiple prior operative

procedures. Clinically there may be a palpable mass or pelvic pain, as well as symptoms relating to the presence of functioning ovarian tissue. In a premenopausal patient with this syndrome, menopausal symptoms fail to occur, and the LH and FSH levels remain in the premenopausal range. Ovarian remnants can be removed at laparotomy or laparoscopy and are often attached by dense adhesions to residual pelvic structures. Residual ovarian tissue may be normal or may be enlarged by the presence of endometriosis or functional cysts. Very rarely a malignancy can develop in an ovarian remnant [134].

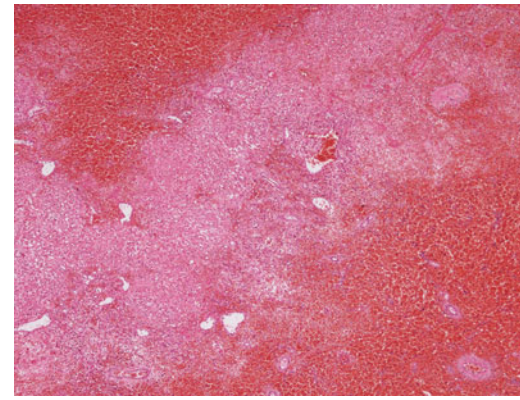

Fig. 2.19 Ovarian torsion. Hemorrhagic necrosis in a hyperstimulated ovary that had undergone torsion

Ovarian Hemorrhage

Ovarian hemorrhage is a common feature in follicular development but is usually minor and intrafollicular or perifollicular. Slight bleeding also occurs with follicular rupture at the time of ovulation, and bleeding also occurs in the vascularization stage of the corpus luteum [3]. Occasionally rupture of a corpus luteum or corpus luteum cyst may result in more severe hemorrhage and hemoperitoneum. This may occur at any time in the reproductive years but especially during pregnancy, and the risk is increased in association with anticoagulant treatment [135]. The right ovary is the source of hemorrhage in two thirds of patients.

Ovarian and Adnexal Torsion

Introduction: Ovarian torsion and hemorrhagic infarction is an uncommon but not a rare event, usually affecting women in reproductive years and is the fifth most common gynecological surgical emergency [136]. In adults, it usually occurs as a complication of an ovarian cyst or benign tumor such as a fibroma or mature cystic teratoma and occasionally a malignant tumor [137–139]. Rarely, particularly in children and young adults, normal ovaries may undergo torsion and infarction [140, 141]. There is also an increased risk of torsion of the normal ovary in pregnancy due to increased motility of the ovary in the first half of human pregnancy [142]. Torsion may also occur in ovarian hyperstimulation syndrome, including hyperreactio luteinalis [143, 144].

The fallopian tube and ovary may undergo torsion as a single unit rotating around the broad ligament, but less commonly the ovary undergoes torsion alone around the mesovarium. The right ovary is most commonly affected as movement of the left ovary is limited by the sigmoid colon [145].

Clinical Features: Clinically the presentation is with acute abdominal pain which may resemble acute appendicitis or with recurrent abdominal pain. Occasionally an adnexal mass is palpable. In children with torsion of a normal ovary, detorsion may be attempted to preserve ovarian function, but in adults excision of the ovary is usually necessary to exclude underlying neoplasia.

Histopathological Findings: Macroscopically the ovary is swollen and dark red. Microscopically there is hemorrhage, edema, and evidence of infarction with often frank necrosis (Fig. 2.19). Multiple blocks should be examined, especially of viable and solid areas at the periphery of the lesion, to determine any underlying cause such as a neoplasm. Complex cystic or papillary patterns raise suspicions of an underlying neoplasm. Necrotic tissue should be examined for the presence of shadows of neoplastic cells, and staining of reticulin fibers may help to identify underlying architecture when cellular detail is obscured.

Massive Ovarian Edema

Massive edema of the ovary is a rare condition affecting young women in their second and third decades and refers to unilateral or rarely bilateral tumorlike enlargement of both ovaries due to accumulation of edema fluid [146–149]. The median age affected is 21 years (range 6–33 years), and most present with abdominal pain and swelling, menstrual abnormalities, and features of androgen excess. The right ovary is affected in >75 % cases, but 10 % cases are bilateral. Partial or complete torsion of the ovary around the mesovarium is seen in 50 % of cases, and partial intermittent torsion that compromises venous and lymphatic drainage has been implicated in the pathogenesis. Management has often been by surgical removal to exclude the possibility of a neoplasm or complete torsion and ovarian ischemia, but conservative management may be possible and lead to resolution.

Macroscopically the ovaries are up to 35 cm diameter (mean 11 cm) with an opaque pearly outer surface, sometimes showing follicular cysts. The cut section is soft and gelatinous and exudes watery edema fluid. Microscopically the ovary shows diffuse edema of the medulla and cortex, although typically the peripheral cortex is spared and shows dense non-edematous collagenous tissue. The stroma is hypocellular and surrounds but does not displace native ovarian structures such as follicles. Clusters of luteinized cells are identified in around 40 % of cases and are thought to be the source of androgen production in patients who have virilization. Small numbers of mast cells, lymphocytes, and macrophages may be scattered within edematous areas. In a minority of cases, there are foci of fibromatous proliferation.

The differential diagnosis includes edematous fibroma and thecofibroma. Sclerosing stromal tumors compress the surrounding ovarian tissue and have a pseudolobular pattern. Metastatic carcinomas including Krukenberg tumors may show severe edema which can resemble massive ovarian edema, and the diagnostic cells may be sparsely distributed; a mucin stain or immunostaining for epithelial markers such as EMA or cytokeratin will establish the correct diagnosis. The relationship between massive edema and ovarian fibromatosis is not yet certain.

Fibromatosis

Ovarian fibromatosis is another cause of ovarian enlargement in young women and refers to overgrowth of collagen producing spindle cells that surround normal follicles. There is a strong clinical overlap with massive ovarian edema. Patients are young (mean 25 years; range 13–39 years), and patients often present with menstrual abnormalities and/or virilization and abdominal pain [148, 149]. There is evidence of torsion in some cases at the time of operation and transitional appearances between the two conditions have led to the suggestion that ovarian fibromatosis and massive ovarian edema represent ends of a spectrum of responses to a single pathogenetic mechanism. It is speculated that local tissue injury stimulates secretion of locally active growth factors that induce massive fibromatous proliferation and/or edema [3]. At operation, the process is bilateral in 20 % cases.

Macroscopically the ovaries are variable in size, sometimes only marginally enlarged, while other cases show ovaries 15 cm in diameter. The external surface may be lobulated or smooth, and the cut surface is typically firm and white or gray, sometimes with small cysts. Microscopically spindle cells surround follicular structures. The fibromatous proliferation is usually diffuse but may be confined to the ovarian cortex; cortical fibrosis is, however, common and this term should be restricted only to those cases where the fibrosis is particularly prominent. The appearances vary from moderate cellularity to a relatively acellular appearance. Luteinized cells may be scattered within cellular and edematous areas. In some cases, there are prominent proliferative features with dense cellularity and numerous normal mitoses, sometimes >20 mitoses per 10 high-power fields [3]. Apparent transition from this cellular form to less cellular collagenous forms has led to the suggestion that this may represent an "immature" form [3].

The differential diagnosis is with a fibroma, which lacks the entrapped normal follicular

structures. Stromal hyperthecosis is also seen in young women and is often bilateral, but it is not associated with acute presentation and does not lead to ovarian enlargement or show abundant collagen production. The more cellular form may be confused with luteinized thecomas which also occur in young women, but these are rare in women aged <20 years and are usually estrogenic and unilateral, while fibromatosis is often bilateral, inactive, or androgenic. Brenner tumors may enter the differential, but the typical epithelial nests should be easily distinguished.

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The Surgery of Ovarian Cancer

3

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Abstract

Surgery has remained the mainstay of treatment for ovarian cancer despite considerable advances in chemotherapy. The findings and results of the initial laparotomy have a greater bearing on the eventual outcome than do many subsequent therapeutic decisions. Hysterectomy with bilateral salpingo-oophorectomy continues to be the most cogent therapy. Both ovaries are removed because of the frequency of bilateral synchronous tumors and the possibility of occult metastases, which may be between 6 and 43 % even in a normal-looking ovary. The uterus is removed because the uterine serosa and endometrium are also frequent sites for occult metastases, and the prevalence of synchronous carcinoma of the endometrium and ovary is relatively high.

Introduction

Surgery has remained the mainstay of treatment for ovarian cancer despite considerable advances in chemotherapy. The findings and results of the initial laparotomy have a greater bearing on the eventual outcome than do many subsequent therapeutic decisions. Hysterectomy with bilateral salpingo-oophorectomy continues to be the most cogent therapy. Both ovaries are removed because of the frequency of bilateral synchronous tumors and the possibility of occult metastases, which may be between 6 and 43 % even in a normal-looking ovary. The uterus is removed because the uterine serosa and endometrium are also frequent sites for occult metastases, and the prevalence of synchronous carcinoma of the endometrium and ovary is relatively high.

The precise role of surgery in the management of the disease remains controversial. Few randomized controlled trials exist and there is a considerable difference of opinion with regard to the surgical effort required. As a result there is substantial national variation in the rates of complete surgical clearance of disease.

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NICE Guidelines

Following the publication and implementation of the national document. Improving Outcomes in Gynecological Cancers in 1999, there was an eventual shift in the management of ovarian cancers to greater specialization, with almost all cancers being treated surgically in regional cancer centers. There has also been a subtle but imperceptible drift to increasing surgical effort brought about in part by the specialization and also the introduction of neoadjuvant chemotherapy.

In April 2011, the National Institute for Health and Clinical Excellence (NIHCE) in the United Kingdom published guidelines regarding the management of ovarian cancer [1]. The publication confirmed that the overall 5-year survival of women with the disease remained less than 35 %, but with the introduction of effective chemotherapy and changes in surgical practice, there had been a twofold increase in survival over the past 30 years.

The guidelines suggest that optimal surgical staging should include a midline laparotomy to allow thorough assessment of the abdomen and pelvis; a total abdominal hysterectomy, bilateral salpingo-oophorectomy, and infracolic omentectomy; biopsies of any peritoneal deposits; and random biopsies of the pelvic and abdominal peritoneum if no disease is obvious and a retroperitoneal lymph node assessment. In early-stage disease, that is, in women with suspected ovarian cancer whose disease appears to be confined to the ovaries, the guidelines suggest that a pelvic lymph node assessment should form part of optimal surgical staging. The guidelines also, however, recommend that surgery should not include systematic retroperitoneal lymphadenectomy (block dissection of lymph nodes from the pelvic side walls to the level of the renal veins) as part of standard surgical treatment.

For advanced disease however the guidelines do not provide any additional support other than confirming that the definition of optimal debulking is no macroscopic residual disease at the end of the procedure.

The guidelines also recommend that a prospective randomized controlled trial should be undertaken to evaluate the therapeutic effect, associated risks, and cost-effectiveness of systematic retroperitoneal lymphadenectomy in those women who appear to have disease confined to the ovary and that research should be undertaken to determine the effectiveness of primary surgery in women with advanced ovarian cancer whose tumor cannot be fully excised. While these are laudable suggestions, their achievement may be difficult. Leaving resectable tumor after chemotherapy may be too difficult a pill to swallow for the majority of gynecological oncology surgeons.

The Surgery of Disease Apparently Confined to the Ovary

The diagnosis of ovarian cancer where there is no disease outside the ovary is difficult. Standard texts opine that optimal treatment should include midline laparotomy, peritoneal washings, a total abdominal hysterectomy, bilateral salpingo-oophorectomy and infracolic omentectomy with peritoneal biopsies, and a retroperitoneal lymph node assessment as noted above. However, in younger women wanting to retain their fertility and in the elderly with significant comorbidity, this approach may not be optimal.

The traditional approach to surgical management is both therapeutic and diagnostic. Surgery is carried out to remove both primary and metastatic disease even when the latter is not visible macroscopically. The diagnostic aim is to upstage the disease to facilitate optimal nonsurgical management. In our series (Hewitt M and Lane G (2006), unpublished data), in apparent stage 1 ovarian cancer, random peritoneal biopsies, removal of the omentum, and lymphadenectomy resulted in an upstaging of the disease in 12 % of 147 cases, 4 % by peritoneal biopsy alone. It is well established that lymph nodes are frequently involved in apparent stage I tumors with Burghardt et al. [2] demonstrating that in his small series of 37 patients, 24 % had positive nodes. Surprisingly, no patients in this study had positive para-aortic nodes without pelvic node involvement. A thorough investigation of the

subject has recently been carried out by Kleppe and colleagues [3]. They demonstrated that in 14 analyzed studies, the mean incidence of lymph node metastases in stages I–II epithelial ovarian cancer was 14.2 % (range 6.1–29.6 %). As expected the incidence of lymph node metastases increased with increasing grade. The lowest incidence (2.6 %) was found in the mucinous subtype and the highest (23.3 %) in the serous subtype. In unilateral tumors pelvic lymph node metastases were found on both sides in 45.2 % of cases, ipsilateral in 38.7 % and contralateral in only 16.1 %. Para-aortic lymph node metastases alone were found in 49.7 % of cases, positive pelvic nodes alone in 20.3 %, and both areas were positive in 29.9 % of the women who had nodal disease. The authors concluded that the incidence of lymph node metastases in apparently early-stage epithelial ovarian cancer is considerable, and the omission of a systematic lymphadenectomy should only be considered in grade 1 mucinous tumors.

Similar data for the omentum and for random peritoneal biopsies are more difficult to find, although in a study with very small numbers, Piver et al. [4] reported an incidence of only 3.2 % in women with apparent stage I disease.

It is clear therefore that for adequate staging, lymphadenectomy is essential. NICE guidelines [1] suggest that this is an untested procedure but agree that it is likely to be more accurate than lymph node sampling with the potential benefit for the woman of avoiding chemotherapy. The guidelines suggest that it is not warranted because of the risk of morbidity and the lack of evidence of a survival advantage.

Fertility-Sparing Surgery for Epithelial Ovarian Cancer

It is inevitable that some women of childbearing age and desirous of children will develop ovarian cancer. It is reported that between 3 and 17 % of ovarian tumors occur in women less than 40 years of age, and these women are more likely to present with earlier-stage and lower-grade disease [5–10]. Over 20 % of epithelial ovarian cancers are diagnosed at stage I [11], and about 7–8 % of stage I cancers occur in women under the age of 35 [12]. Provided that the preoperative imaging is suggestive of a malignant ovarian lesion with no disease outside the ovary, under these circumstances limited surgery might be attempted. This would include a unilateral salpingo-oophorectomy, bilateral pelvic lymphadenectomy, para-aortic lymphadenectomy, omentectomy, and peritoneal biopsies. The rationale for the lymphadenectomy is outlined above. Provided no disseminated disease is discovered and the disease is stage 1a, no further treatment should be necessary unless the histological subtype is unfavorable. This is discussed elsewhere in this book.

Fertility-sparing surgery such as this cannot of course identify all sites of metastatic disease and recurrence in a proportion of cases is inevitable. Numerous retrospective studies have investigated whether less than radical surgery can preserve fertility without compromising survival. Schilder and her colleagues [13] collated multi-institutional data on 52 patients with invasive stage IA or IC disease. Forty-two patients had stage IA disease and 10 stage IC. Twenty-five patients (48 %) had mucinous tumors, 10 (19 %) serous, 10 (19 %) endometrioid, 5 (10 %) clear cell, and 2 mixed. Nineteen patients, 11 with stage IA and 8 with IC tumors, received adjuvant chemotherapy with a platinum-containing agent and paclitaxel. Five patients (10 %) developed recurrence between 8 and 78 months following initial surgery and two died of disease within the study period, both mucinous tumors, but overall there was no statistically significant relationship between tumor substage or tumor histology and the frequency of tumor recurrence. It is interesting to note that 3 of the 5 recurrences were in the contralateral ovary. Following treatment 24 patients attempted pregnancy and 17 (71 %) conceived. Six of the 17 had received chemotherapy.

Park et al. [14] looked at women of all stages who underwent fertility-sparing surgery. Of the 62 epithelial ovarian cancers studied, 36 were stage IA, 2 were stage IB, 21 were stage IC, and one each were IIB, IIIA, and IIIC. Forty-one tumors were mucinous, 8 were endometrioid, 7 were serous, 4 were clear cell, and 2 were

mixed. Forty-eight tumors were grade I, 5 were grade II, and 9 were grade III. In this study as well as a standard staging procedure as in the previous study, 17 patients also had wedge biopsies of the other normal-looking ovary. Full staging was not carried out on each patient; however, all underwent partial omentectomy, multiple peritoneal biopsies, and washings. Pelvic and para-aortic lymphadenectomy and appendicectomy were optional. Patients with early-stage disease and high-risk factors including high-grade lesions, clear cell histological type, tumor growth through the capsule, surface excrescences, malignant cells in ascites or peritoneal washings, preoperative rupture, and dense adhesions were treated with platinum-based chemotherapy. Regular clinical follow-up was complemented by tumor marker estimation and imaging by ultrasound, computed tomography, magnetic resonance imaging, and positron-emission tomogram. Eleven cancers recurred, 7 of them mucinous tumors 5 of which were also stage I. Two stage 1A clear cell tumors also recurred and 1 stage 1C endometrioid cancer. Two of the patients who were upstaged to stages II and III due to microscopic disease had recurrence of their tumor and died of their disease 10 and 16 months after initial treatment. Of the 19 patients who attempted to conceive, 8 had received Carboplatin and Paclitaxel chemotherapy and 5 of these managed to conceive. Fifteen patients in total became pregnant and no congenital abnormalities were reported. The authors concluded that fertility-sparing surgery is effective but emphasizes the need for accurate surgical staging and that careful attention is paid to grade and histological type. In this study only 4 patients had clear cell cancers, but 2, both with stage IA disease, recurred and one died; in addition, two-thirds of women with grade III tumors also recurred. Otherwise recurrence rates were low and pregnancy was easy to achieve even after chemotherapy.

Wright and his colleagues [15] reported similar conclusions. Using the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) data, women under 50, diagnosed with epithelial ovarian cancer stages 1A to 1C, were studied [16]. In total 1,186 women with serous, endometrioid, mucinous, and clear cell tumors were studied. The study concluded that neither uterine nor ovarian preservation had an adverse impact on survival but did not formally address the impact of grade or histological subtype. The small study of Schlaerth and colleagues [17] also confirmed the safety of fertility-sparing surgery compared with the conventional approach but again was underpowered to address tumor grade and histological subtype.

Kajiyama and colleagues [18] analyzed data from multiple institutions with regard to survival. In their study no difference was found in disease-free survival and overall survival between women 40 years of age or under who underwent fertility-sparing surgery and those both under and over 40 who underwent radical surgery regardless of the stage I substage and whether there was intraoperative capsule rupture. In contrast stage IC attributable to preoperative rupture, positive ascites, or peritoneal washings appeared to be a contraindication to fertility-sparing surgery as did grade III and clear cell histology. Vergote et al. [19] concluded that mucinous histology yielded the most favorable prognosis in early-stage ovarian cancer.

During surgical staging the value of wedge biopsy of the other ovary remains unclear. It has been reported that wedge biopsy may result in mechanical infertility [20] or ovarian failure [21]. The incidence of occult metastases in the unaffected ovary is unclear. Some studies report an incidence of between 7 and 12 % in normal-looking ovaries [22, 23]. Later reports found that none of their patients who underwent fertility-sparing surgery had microscopic metastases on routine biopsies of their macroscopically normal-looking ovaries [24, 25]. Another report suggested only 2.5 % of contralateral ovaries are affected [26]. It is therefore unclear whether random biopsies are helpful or harmful, although the incidence of malignancy in 2 of 9 benign-looking cysts in the study of Park et al. [14] suggests that there should be a high index of suspicion when ovarian appearances deviate from normal.

Some studies have reported a high incidence of endometrial abnormalities in women with

endometrioid ovarian cancer and recommend that in such cases, an endometrial assessment should be undertaken [27]. There is little follow-up data to support the reported incidence of 14 % of women with stage I endometrioid ovarian cancer suffering from endometrial cancer. Endometrial sampling was carried out in 70 % of the patients undergoing fertility-sparing surgery in the study by Schlaerth and colleagues [17], but no endometrial abnormalities were found, although one patient developed an endometrial cancer 15 months postoperatively. Zaino et al. [28] discovered that 10 % of women with endometrioid ovarian cancer also had a secondary or synchronous carcinoma of the endometrium at the time of surgery. Endometrial assessment should therefore form part of the assessment when fertility-sparing surgery is considered.

At the end of childbearing, completion surgery with oophorectomy or hysterectomy should be considered. In the study of Schilder and colleagues [13], 3 of the 5 recurrences occurred in the contralateral ovary. Similarly, Morice et al. [22] found 5 of 7 recurrences in the retained adnexa. Whether completion surgery affects long-term survival is unknown, and thus the decision to undergo surgery should be individualized.

The Surgery of Germ Cell Tumors of the Ovary

Ovarian germ cell tumors account for 20–25 % of all ovarian neoplasms but only 3 % of these are malignant [29]. The age distribution of the tumors shows a sharp peak between 15 and 19 years to which teratomas and dysgerminomas contribute [30] and a secondary wider peak at ages 65–69 composed mainly of teratomas. Historically, the women and children had a poor prognosis when treated with surgery alone, but the introduction of radiotherapy and combination chemotherapy with vincristine, actinomycin D, and cyclophosphamide improved the outcome considerably [31]. Later with the addition of platinum-based regimens, a further improvement in survival rate was accomplished [32].

The preponderance of these tumors in the second and third decades of life results in decisions concerning childbearing and probabilities of recurrence. Fortunately, developments in chemotherapy have dramatically improved the prognosis for many patients who develop the more aggressive types of germ cell tumor. This permits reduced surgical aggressiveness without diminishing survival [33]. The standard surgical treatment including hysterectomy and bilateral salpingo-oophorectomy with omentectomy, lymphadenectomy, and biopsies may by safely limited to staging plus adnexectomy without significant reduction in survival rate in women who wish to retain fertility.

As with epithelial ovarian cancers, comprehensive staging is important to allow individualization of treatment. The retrospective study of Palenzuela and colleagues [34] reported on 60 patients between the ages of 0.4 and 27.9 years. Fifty-two patients presented with abdominal pain or a mass, 4 with premature puberty and 2 with dysmenorrhea. Two patients had surgery after being followed-up for gonadal dysgenesis and were suspicious for gonadoblastoma. Ten patients were in a poor prognosis group because of high levels of the tumor markers alpha-fetoprotein (AFP) and human chorionic gonadotrophin (HCG) at diagnosis. Fifty-eight patients underwent primary surgery, which included midline laparotomy, peritoneal cytology, thorough exploration of the abdomen and pelvis with lymph node biopsies, and biopsies of the contralateral ovary if necessary in addition to adnexectomy and complete intact resection with enucleation of the contralateral lesion if the tumor was bilateral. Tumors were designated Ia, Ib, or Ic if staging information was complete and Ix if not. The most common histological groups were mixed tumors most of which included yolk sac tumors and some immature teratomas. Dysgerminomas were the next most common histological type. Among the stage I tumors, 24 were observed and not given chemotherapy: None of the 8 stage Ia tumors relapsed, all stage Ic tumors relapsed as did 5 of the 13 stage Ix tumors. None of the stage Ic or Ix tumors receiving adjuvant chemotherapy relapsed. Adequate

Table 3.1 Survival according to stage in ovarian germ cell tumors

Stage	No	6 year survival %
I	41	95.1
II	16	93.8
III	58	97.3
IV	16	93.3

Table 3.2 Conception following treatment for ovarian germ cell tumors

Conception outcome	FIGO stage				Total
	I	II	III	IV	
Conceived	20	1	8	0	29
Did not conceive	7	0	2	0	9
Total	27	1	10	0	38

staging therefore may, if no disease is discovered outside the ovary, avoid chemotherapy. The authors concluded that the rate of improper surgical staging significantly increased with surgeons less experienced in gynecological surgery, and the risk of relapse in stage I tumors undergoing observation increased significantly if the tumor was improperly staged. These relapses did not appear to modify survival because chemotherapy salvaged patients after relapse.

Current platinum-based chemotherapy regimens produce excellent survival figures, even for advanced-stage disease. This study demonstrated a 93.3 % 6-year survival for stage IV tumors (Table 3.1).

To avoid the use of adjuvant chemotherapy, it is of prime importance to remove the ovarian lesion intact. This precludes the use of ovarian biopsy in stage I tumors and mandates the use of midline laparotomy to optimize the chances of intact removal of the lesion. Laparoscopic surgery therefore appears to be contraindicated.

When an ovarian lesion is removed inadvertently and intact but not staged, further staging information might be achieved laparoscopically and a negative result obviates the need for chemotherapy. The results of adjuvant chemotherapy are so good, however, that in more advanced disease, stage Ic and above, chemotherapy will be indicated in any event and further surgery might be deemed unnecessary.

Following treatment the reproductive outcome of women after conservative surgery is excellent. In the series reported by Tangir et al. [35], fertility-sparing surgery was performed in 64 of 106 patients with malignant germ cell tumors. In the study, 38 women attempted conception and 29 (76 %) achieved at least one pregnancy. Among these were 10 patients with advanced disease who were treated conservatively and of these 8 also conceived (Table 3.2). Among the women who conceived, 25 of the 29 had received combination chemotherapy.

Low et al. [36] reported similar figures. Seventy-four women underwent conservative surgery for malignant germ cell tumors of the ovary, 47 (64 %) received adjuvant chemotherapy, and of the 20 of these who attempted conception 19 succeeded.

Of 81 similar patients studied by Zanetta et al. [37] who underwent conservative surgery, 47 received adjuvant chemotherapy, 20 attempted to conceive, and 19 were successful. All 12 who did not receive chemotherapy conceived. Gershenson [38] published similar data with 16 of the 40 patients in his study attempting conception. Nine conceived naturally and 3 more conceived after fertility treatment. It was not stated how many of the 10 patients with advanced disease conceived. Research from the same authors [39] showed that 3 of 16 women who underwent conservative surgery for dysgerminoma and who were subsequently treated with bleomycin, etoposide, and cisplatin conceived.

For germ cell tumors, conservative surgery is therefore a realistic option in women desiring fertility even if chemotherapy is required.

The Surgery of Sex Cord-Stromal Tumors of the Ovary

This category of ovarian tumors includes all those that contain granulosa cells, theca cells and their luteinized derivatives, Sertoli cells, Leydig cells, and fibroblasts of gonadal stromal origin. The tumors are rare and represent approximately 7 % of all ovarian malignancies [40].

Because of their rarity the surgical management of these tumors is not well defined. Juvenile granulosa cell tumors are generally unilateral and may occur at any age, but in prepubertal girls 82 % present with isosexual pseudoprecocity [41]. Adult granulosa cell tumors are frequently found in postmenopausal women but often occur at puberty. In the postmenopausal group, surgery has generally reflected that of epithelial ovarian cancer including full surgical staging to involve omentectomy, pelvic and para-aortic lymphadenectomy, and biopsies [42, 43]. Older women and those with advanced or bilateral ovarian disease may benefit additionally from hysterectomy and bilateral salpingo-oophorectomy although there is little data to support this approach. Because the majority of tumors are unilateral, in younger patients desiring fertility and with stage Ia disease, which has an excellent prognosis [41], unilateral salpingo-oophorectomy seems appropriate.

Recent studies of both granulosa cell and Sertoli-Leydig tumors strongly suggest that lymph node metastases are rare, and therefore the use of routine lymphadenectomy is likely to produce little additional information and may be omitted from the primary or secondary staging procedure [44, 45]. This was confirmed by Thrall et al. [46] who also found in her study of 87 patients that 8.5 % of those with granulosa cell tumors were also found to have a concurrent endometrial carcinoma. An endometrial assessment is therefore essential in women with this tumor and in whom fertility-sparing surgery is considered.

Secondary surgical staging in women who have undergone ovarian cystectomy or unilateral salpingo-oophorectomy is likely to remain an important management tool. In Thrall's series (2011), of 8 women who had secondary staging 2 had residual disease, one in the residual ovary. This is similar to the series of Brown et al. [45] where 4 women had disease in residual ovarian tissue. In Thrall's series 6 of the 10 women found to have disease at secondary staging had only microscopic extraovarian disease confirming the importance of this procedure even if lymphadenectomy is not required.

The Surgery of Borderline Ovarian Tumors

There is clear evidence that there is a group of epithelial ovarian tumors whose histology and behavior falls between benign and frankly malignant ovarian neoplasms. Borderline tumors represent between 10 and 20 % of all epithelial ovarian malignancies [47]. Approximately 80 % of women have stage I disease [48], and the median age at diagnosis is 40 compared to about 60 for women with invasive carcinoma [49, 50].

Serous borderline tumors are bilateral in a third of cases [50, 51]. They are usually associated with noninvasive implants, but invasive implants occur in 20–25 % of cases [52]. Bilateral tumors and noninvasive implants do not predict a worse outcome compared with those with invasive implants [53, 54]. Invasive implants may progress to invasive carcinoma whereas noninvasive implants will remain stable and regress after removal of the main ovarian tumor [55]. Women with serous tumors without invasive implants have a 10-year survival of 95 % compared with 60–70 % in women with invasive implants [49]. Women with invasive implants develop progressive disease in about 30 % of cases while only 2 % of women without invasive implants will eventually progress [56, 57].

Mucinous tumors are either of intestinal or endocervical type [48, 54]. Tumors of intestinal type may be very large and are nearly always unilateral. In the case of bilaterality, it is important to look for a primary intestinal tumor. The endocervical subtype may be bilateral. Both subtypes may present with intraepithelial carcinoma and microinvasion of less than 10 mm^2. Extraovarian spread is infrequent and nearly always as pseudomyxoma peritonei although most of these represent dissemination from a mucinous tumor of the appendix. An appendicectomy should therefore be carried out at primary surgery for an intestinal borderline tumor. All mucinous borderline tumors have the potential to recur as invasive adenocarcinoma. This is particularly true if ovarian cystectomy rather than salpingo-oophorectomy has been performed [58].

The diagnosis of borderline ovarian tumor is difficult to make before surgery. It is also difficult

to distinguish between serous and mucinous tumors [59]. Intraoperative frozen section diagnosis is not helpful for the discrimination between borderline tumors and epithelial ovarian cancer when underdiagnosis of up to 25 % has been reported [60, 61]. It is even more difficult to determine preoperative variables that might influence prognosis and therefore alter the surgical approach accordingly. FIGO stage is the strongest prognostic factor for recurrence and survival. Micropapillary histology has been reported as an additional risk factor, but this is a postoperative diagnosis as well as being controversial as the poor prognosis is seen only with invasive implants [62–65]. In the retrospective study from the Norwegian Radium Hospital [51], by multivariate analysis the only three independent risk factors for disease-free and long-term survival were stage, histologic type, and age. Using DNA ploidy the women could be divided into risk groups with low risk being characterized by stage I disease, diploid phenotype, and age less than 40. The high-risk group who had a greater than 75 % chance of dying of the disease had aneuploid tumors stage II and III disease and age older than 70. Lymph node involvement has not been shown to be an independent risk factor. It is worthy of note that tumors with less than 10 mm^2 of invasion behave as borderline tumors and are classified as such [66–68]. Surgery is therefore essential before a diagnosis is made.

The standard surgical approach for the treatment of borderline ovarian tumors is therefore the same as for epithelial ovarian cancers including hysterectomy, bilateral salpingo-oophorectomy peritoneal biopsies, and omentectomy with appendicectomy in mucinous borderline ovarian tumors. The involvement of lymph nodes, even if the disease is upstaged as a result, appears not to influence survival [51, 69]. Lymph nodes are rarely involved with mucinous borderline tumors and lymphadenectomy may be omitted, as there is no difference in recurrence or survival rates [70].

Nearly one-third of women with borderline ovarian tumors are under 40, and therefore the preservation of reproductive function may be desirable and feasible. Relapse rates are higher following ovarian cystectomy (12–58 %) compared to bilateral salpingo-oophorectomy (0–20 %) and radical surgery (2.5–5.7 %). Multifocality may be a reason why cystectomy fails to control the disease; extensive sampling of the margin of the tumor is important [21, 24] as involved margins are also strong predictors of recurrence [71].

Many women are referred to gynecological oncology centers following the diagnosis of a borderline tumor following surgery for apparent benign disease. Staging is therefore incomplete. Occasionally surgery for a borderline tumor will unearth an unexpected malignancy when the abdomen has not been thoroughly explored. Snider et al. [72] discovered in his small series that none of 12 patients with mucinous tumors were upstaged while 4 of 13 serous tumors were upstaged although the recurrence and survival advantages were not discussed. As stage is an important prognostic indicator, the restaging of serous tumors appears worthwhile.

Early detected recurrences are curable with repeated surgery, and therefore further conservative surgery may be considered in young women wishing to retain fertility [48, 65, 70]. If invasive implants are diagnosed, this should not be recommended as women suboptimally debulked have poor survival [73].

Follow-up of borderline ovarian tumors should be lifelong as recurrences may develop after more than 15 years. In conservatively treated women, close follow-up of the remaining ovary or ovaries is essential. It is not clear whether the remaining ovary and uterus should be removed after completion of the family. For low-risk borderline ovarian tumors, this is probably unnecessary; however, it should be given strong consideration in high-risk disease. The alternatives are to await recurrence or treat radically.

The Surgery of Advanced Ovarian Cancer

NICE guidelines [1] are clear that the aim of surgery in the management of advanced, stage II–IV, ovarian cancer should be the complete resection

of all macroscopic disease [1]. Previously, the definition of optimal surgical debulking had been set at less than 2 cm residual disease, which was open to interpretation. The objective is now clear.

The spread of ovarian cancer occurs directly to adjacent tissues, through the lymphatic system and throughout the peritoneal cavity where surface deposits may be seen on all areas of the gastrointestinal tract and the omentum and peritoneal surfaces including those of the liver, spleen, and diaphragm, sometimes invading from the surface into these organs. Until recently the standard of care for all patients with advanced ovarian cancer particularly FIGO stages III and IV has been primary debulking surgery followed by Paclitaxel and Carboplatin chemotherapy [74]. However, no prospective randomized controlled trials are available, proving that primary debulking surgery improves the prognosis of patients with ovarian cancer.

The management of advanced ovarian cancer began to change soon after the publication of van der Burg and colleagues [75] who reported on 319 patients who had residual lesions greater than 1 cm after primary surgery. After three cycles of cyclophosphamide and cisplatin, the patients were randomized to undergo further debulking surgery or no surgery followed by three further cycles of chemotherapy. The authors concluded that debulking surgery significantly lengthened progression-free and overall survival. These findings were confirmed by the EORTC [76] who also concluded that neoadjuvant chemotherapy followed by interval debulking surgery was not inferior to primary debulking surgery followed by chemotherapy as a treatment option for patients with bulky stage IIIC or IV ovarian carcinoma. The study recorded a higher morbidity and mortality after primary debulking when compared with interval debulking surgery. Using an intention to treat analysis, the hazard ratios for death and progression comparing neoadjuvant chemotherapy to primary debulking was 1.01 and 0.98, respectively. Therefore to reduce morbidity and mortality, in addition to achieving the goal of optimal cytoreduction that is no macroscopic residual disease, this approach has received widespread acceptance. Image-guided core biopsies are now standard practice for diagnosis [77].

It has been long reported that survival when residual disease exceeds 2 cm diameter is poor [78]. The inability to optimally debulk has often been attributed to adverse tumor biology rather than lack of surgical effort; however, the variations in optimal debulking rates between countries such as the United Kingdom where the average optimal resection rate was only 42.9 % after neoadjuvant chemotherapy while in Belgium optimal debulking was achieved in 62.9 % of patients at primary surgery [76].

Numerous studies although retrospective in nature strongly suggest that the effort employed by the surgical team will reap benefits in terms of survival although mortality and short-term morbidity may well increase. The role of lymphadenectomy is unclear, although unlike endometrial cancer there are no reports of the procedure influencing survival in an adverse manner. Rouzier et al. [79] used SEER data to investigate the effect of lymphadenectomy on survival. The group found a beneficial effect of lymphadenectomy in epithelial ovarian cancer regardless of stage but acknowledged potential biases in their methodology including stage migration and incomplete data requiring estimation of disease extent. They also suggest that a thorough lymphadenectomy might reflect the quality of cytoreductive surgery.

Chi et al. [80] compared aggressive upper abdominal cytoreduction with earlier and less aggressive primary cytoreduction. They noted an increase in 5-year progression-free survival and overall survival from 14 to 31 % and 35 to 47 %, respectively. Peiretti and coworkers [81] reported on their experience with extensive upper abdominal surgery including diaphragm peritonectomy, splenectomy, distal pancreatectomy, partial liver resection, cholecystectomy, and gastric resection. Their findings were similar and demonstrated progression-free and overall survival figures of 19.9 and 57.6 months, respectively.

Other surrogates of maximal surgical effort including transverse colectomy [82] demonstrated median survival figures of 68.3 months. In addition Aletti and colleagues [83] showed an

improvement in survival in a subgroup of women with less than 1 cm residual disease following cytoreductive surgery when diaphragm resection was performed. This was associated with an improved survival from 28 % in those without diaphragm resection to 55 % in those where this was carried out.

Surgery for stage IV disease has also been considered futile. However, complete resection is a clear positive prognostic factor [84]. Aletti et al. [85] demonstrated an improved survival in women with stage IV disease with positive pleural cytology when the disease was optimally debulked in the abdomen. Rafil et al. [86] also showed that maximal debulking after neoadjuvant chemotherapy was associated with improved survival compared with those women with stage IV disease that were suboptimally debulked.

Therefore although there is no randomized data to confirm that maximal surgical effort results in improved outcomes, there is a groundswell of opinion which is leading the specialty in this direction.

Secondary Debulking Surgery

Routine second-look procedures have largely fallen out of favor. Secondary debulking in the presence of radiologically proven recurrence continues to hold a small but significant place in the management of advanced ovarian cancer although again there are few randomized trials to provide evidence of its efficacy.

Patients who are platinum sensitive, do not have ascites or intestinal tumor involvement, have tumor outside the upper abdomen, and have serous histology have significantly higher tumor resection rates [87]. It is probable, however, that only women who are cytoreduced to no visible disease are likely to obtain a survival advantage. An additional predictive factor of a good surgical response is a preoperative CA125 of less than 250 U/ml [88]. This does not of course preclude palliative surgery for symptom control when necessary, but it is imperative that patients undergoing surgery under these circumstances are aware of the possible morbidity without necessarily achieving prolongation of life, although increased quality is the end point under these circumstances.

Risk-Reducing Surgery

The group most likely to benefit from prophylactic oophorectomy are those women who have a particular genetic risk and those in a number of familial cancer syndromes. The lifetime risk of development of ovarian cancer has been estimated at 60 % in BRCA1 carriers and 27 % in BRCA2 carriers [89–91]. Later reports suggest that these may be overestimates [92, 93].

It is reported that the age of development of ovarian cancer in women with two or more relatives affected with the disease is younger than the median age of onset in the general population [94]. In this study, the median age at diagnosis was 47 years, 14 years earlier than the median age of sporadic ovarian cancer diagnosis in the United States. Another study examined apparent site-specific ovarian cancer, hereditary breast ovarian cancer syndrome, and Lynch II families [95]. The hereditary breast ovarian cancer syndrome patients were diagnosed on average 7 years younger than the general population mean of 59 years. Apparent site-specific family members were diagnosed 10 years younger while the Lynch II family members were diagnosed 14 years earlier than the general population mean.

There appears to be no correlation between age at presentation with ovarian cancer affecting mother and daughter and therefore probably with other relatives, but there is a relationship between sisters which should be taken into consideration when planning surgery [96].

In the United States a consensus panel has recommended prophylactic oophorectomy for a woman in a family with hereditary ovarian cancer syndrome at age 35 or when she has completed childbearing [97].

Once a genetic link has been established, the technique of oophorectomy is relatively simple. The ovaries with the Fallopian tubes may be removed through either a small low transverse

abdominal incision or more commonly laparoscopically using three or four appropriately placed 0.5–1 cm abdominal incisions [98, 99]. With the laparoscopic approach, a 24–48 hours hospital stay is usually required but may be a day case procedure with a recovery period of a few weeks.

A report by Colgan et al. [100] suggested that peritoneal lavage at the time of prophylactic oophorectomy is likely to identify occult malignancy, and the authors have recommended that this procedure is carried out whenever such surgery is performed.

Women in low-risk groups in their 40s are among the most likely to suffer from benign gynecological conditions such as prolapse, urinary incontinence, and menstrual disturbance all of which may lead to pelvic surgery. At the time of this surgery, the ovaries may be removed.

The lifetime risk of the development of ovarian cancer is widely variable throughout the world and in the United Kingdom is 1.4 %. Several studies have shown that carrying out prophylactic oophorectomy at the time of incidental benign abdominal or pelvic surgery can prevent a proportion of these cases. For example, Rozario and colleagues [101] analyzed data from 404 patients with ovarian cancer and demonstrated that if oophorectomy had been carried out on everyone who had undergone pelvic surgery over the age of 40, 10.9 % of ovarian cancers would have been avoided. This figure reduced to 6.7 % if the surgery had been carried out over 45 years and to 4 % if over 50 years.

There is now compelling evidence that serous tubal intraepithelial carcinoma (STIC) arising from the tubal fimbriae is a precursor of high-grade serous ovarian carcinoma of the ovary and possibly the peritoneum [102], and standardization of morphologic and immunohistochemical reproducibility of diagnosis is being devised [103]. The removal of the Fallopian tubes at the time of benign gynecological surgery is becoming noticeably more common and has been recommended for both low- and high-risk groups [104]. It remains to be seen whether the incidence of serous ovarian cancer reduces as a result, and further large-scale studies are required.

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Advances in the Medical Management of Ovarian Cancer

4

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Abstract

The term ovarian cancer includes a family of diseases with distinct anatomical, morphological, and genetic distinctions. The term is used to generally encompass tumors that are thought to arise from the ovary and the fallopian tube and also primary peritoneal carcinoma. The disease carries a poor prognosis with outlook primarily being dictated by the anatomical stage of disease at presentation. A recent analysis of the Surveillance, Epidemiology, and End Results database for US patients presenting with epithelial ovarian carcinoma between 1995 and 2007 has demonstrated a relative 10-year survival of 84 %, 59 %, 23 %, and just 8 % for FIGO stages 1, 2, 3, and 4, respectively.

Introduction

The term ovarian cancer includes a family of diseases with distinct anatomical, morphological, and genetic distinctions. The term is used to generally encompass tumors that are thought to arise from the ovary and the fallopian tube and also primary peritoneal carcinoma. The disease carries a poor prognosis with outlook primarily being dictated by the anatomical stage of disease at presentation. A recent analysis of the Surveillance, Epidemiology, and End Results database for US patients presenting with epithelial ovarian carcinoma between 1995 and 2007 has demonstrated a relative 10-year survival of 84 %, 59 %, 23 %, and just 8 % for FIGO stages 1, 2, 3, and 4, respectively [1].

After 15 years of stagnation where ovarian cancer was considered to be a single disease treated uniformly by primary surgery and followed where appropriate by platinum-based chemotherapy, there has, over the past 5–10 years, been a revolution in our understanding of the biology of ovarian cancer. Coupled with the improved understanding of ovarian cancer biology, the molecular revolution has resulted in

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the development of a range of potential therapeutics to target the specific molecular aberrations identified within the disease that we know as ovarian cancer. As we enter 2014, we have already begun to see improvements in the management of ovarian cancer with one licensed targeted molecular agent with activity in first-line, platinum-sensitive, and platinum-resistant relapse. The results of phase 2 and phase 3 clinical trials are encouraging, and a series of other molecules are looking promising and are likely to be licensed for use in the clinic in the relatively near future. Further spectacular improvements are expected through better exploitation of the biological differences between subtypes of tumors whether defined histologically or by molecular parameters.

Current Thinking Concerning the Pathogenesis of Ovarian Cancer

It is becoming increasingly clear that while ovarian cancer, fallopian tube cancer, and primary peritoneal cancer have historically been treated as a single disease, this is now inappropriate. In terms of morphology, a retrospective analysis of 8,704 patients with FIGO stages 3 and 4 ovarian cancer, enrolled into 7 Gynecologic Cancer InterGroup (GCIG) randomized trials of chemotherapy, demonstrated that progression-free (PFS) and overall survival (OS) were significantly worse for patients with clear cell or mucinous ovarian cancer when compared with the more common serous subtype of disease [2]. The analysis was controlled for study, stage, age, and presence of residual disease and demonstrated a hazard ratio for time to progression of 2.1 for mucinous and 1.6 for clear cell; for death, the corresponding hazard ratios were 2.7 and 2.2, respectively. The median OS for serous histology was 40.8 months and for clear cell and mucinous carcinomas were 21.3 and 14.6 months, respectively. For the other histological subtypes (adenocarcinoma NOS, endometrioid, mixed cell carcinoma, undifferentiated carcinoma, and the small group of transitional cell carcinomas), the outcome did not differ significantly from that seen for serous carcinomas.

Development of a Binary Classification of Ovarian Cancer

Based on clinicopathological and more recent molecular studies, it is now proposed that ovarian cancer be divided into two broad categories, designated type 1 and type 2 [3]. Type 1 cancers are the low-grade neoplasms such as low-grade serous, but also encompassing low-grade mucinous, endometrioid, clear cell carcinomas, and malignant Brenner tumors. These neoplasms comprise 25–30 % of ovarian cancers; they tend to present at an early stage and behave in a relatively indolent fashion. Type 2 cancers, on the other hand, comprise approximately 75 % of all epithelial ovarian malignancies; they behave in a much more aggressive fashion and usually present at advanced stage. These comprise high-grade serous, high-grade endometrioid, and undifferentiated carcinomas, including carcinosarcoma. There are distinct molecular genetic differences between type 1 and type 2 ovarian carcinomas, and it may be possible to exploit these differences with novel therapeutics.

Molecular Phenotype of Ovarian Carcinoma

Tothill et al. [4] performed microarray gene expression profiling on 285 serous and endometrioid tumors of the ovary, fallopian tube, and peritoneum. They identified 6 distinct molecular phenotypes: 2 represented low-grade serous and endometrioid carcinoma; both of these subtypes had an excellent prognosis and would equate to type 1 ovarian carcinoma; the remaining 4 subtypes (which would be included among type 2 cancers) represented high-grade and advanced stage cancers of serous and endometrioid morphology. These subtypes were termed "differentiated," "immunoreactive," "mesenchymal," or "proliferative." The mesenchymal subtype appeared to carry a particularly poor prognosis.

Type 1 Tumors

Type 1 tumors tend to be more stable genetically, and there are distinct gene mutations associated with the various histological subtypes. Low-grade serous carcinomas express KRAS, BRAF, and ERBB2 mutations in approximately two thirds of cases. Low-grade endometrioid carcinomas have aberrations in the WNT signaling pathway, as well as PTEN and PIK3CA. Mucinous carcinomas express KRAS mutations in more than 50 % of cases. Clear cell carcinomas, although classified as type 1 cancers, do not fit happily into this broad classification. Patients who present with advanced stage ovarian clear cell carcinomas tend to have disease that is relatively resistant to chemotherapy and which carries a poor prognosis. On the other hand, some patients present with early stage clear cell carcinoma which seems to behave in a much more indolent fashion, and some have questioned whether adjuvant therapy is even necessary for this group [5, 6]. The molecular biology of clear cell carcinoma has been studied in some detail recently and has been reviewed by Tan et al. [7]: mutations in ARID1A are described in 40–57 % of clear cell carcinomas; IL6 STAT3 HIF upregulation leading to IL6 overexpression is described in 49 %, HNF-1 beta upregulation in almost 100 %, TMS/1/ASC methylation in 69 %, and PI3K/AKT/mTOR pathway activation by either PTEN loss (40 %), PIK3CA mutation (33 %), or AKT amplification (14 %). HER-2 amplification or overexpression is also seen in 14 %, PPM1D amplification in 10 %, and loss of mismatch repair genes (hMLH1 and hMSH2) in between 7 and 18 %.

Type 2 Tumors

Type 2 ovarian carcinomas comprise predominantly high-grade serous cancers, although undifferentiated carcinoma and malignant mixed mesodermal cancers are included in this group. The high-grade serous cancers are characterized by P53 gene mutations in more than 80 % of cases and do not show the pattern of mutations seen in type I ovarian carcinoma. Although relatively few malignant mixed mesodermal tumors have been studied, there appears to be a high incidence of P53 gene mutation in this group as well [3]. Subsequent work by the Cancer Genome Atlas Project [8] has analyzed 498 high-grade serous ovarian carcinomas in detail and has demonstrated that P53 gene mutation occurred in at least 96 %. A fascinating finding was that BRCA 1 or BRCA 2 was found to be mutated in 22 % of tumors owing to a combination of germline and somatic mutations. This figure is substantially in excess of the 5–10 % of patients where we have historically believed that BRCA 1 or BRCA 2 gene mutation has been implicated in the pathogenesis of their ovarian cancer. Pathway analysis suggested that approximately 50 % of high-grade serous carcinomas had abnormalities in homologous repair mechanisms. This is a particularly important finding with potential therapeutic implication for targeting by PARP inhibitors.

The clinical significance of classifying serous ovarian carcinoma into a two-tier system of high-grade and low-grade ovarian carcinomas using conventional morphological criteria has also been demonstrated to be relevant in a retrospective study of 241 patients with the serous subtype of ovarian carcinoma entered into the GOG protocol 158 [9] and treated with the combination of carboplatin and paclitaxel. A panel of pathologists reclassified the tumors in a blinded fashion based on an assessment of nuclear atypia, with mitotic rate used as a secondary feature. Twenty-one patients were classified as low grade and 220 as high grade. There was a close association between the two-tier system and conventional FIGO grading (grade 1 vs. grades 2 and 3 combined) with agreement in 230 of the cases (95 %). Patients with low-grade tumors had significantly longer PFS (45 vs. 19.8 months, respectively) and OS (126.2 vs. 53.8 months, respectively) [10].

Verhaak et al. [11] used the catalogue of the Cancer Genome Atlas Project to develop a supervised prognostic signature based on 193 genes developed from 215 expression profiles. They subsequently went on to validate this in 524 expression profiles and were able to identify a better prognosis group with a median survival of around 48 months and a group with a worse prognosis with a median survival of approximately

36 months. Using the full catalogue of the Cancer Genome Atlas Project together with the associated clinical data, they went on to expand the description of the previously described differentiated, immunoreactive, mesenchymal, and proliferative subtypes of ovarian cancer by integrating subtype and prognostic classifiers into a set of 489 expression profiles and developed a prognostic framework termed Classification of Ovarian Cancer (CLOVAR). They subsequently went on to test its accuracy in predicting outcome on a data set of 879 publicly available expression profiles of high-grade serous ovarian carcinoma. Using this methodology, they were able to identify a poor prognostic group representing 23 % of cases with a median survival of 23 months and a platinum resistance rate of 63 %, compared with the rest of the cases where the median survival was 46 months and the platinum resistance rate 23 %. It was possible to further optimize the model by associating the outcome prediction model with the BRCA1 and BRCA2 mutation status, residual disease after surgery, and disease stage.

Future research work and translational work attached to the current raft of clinical trials will no doubt begin to assess treatment efficacy and outcome data within the distinct molecular phenotype of the disease, and ultimately clinical trials will need to be developed specifically for patients with a specific molecular profile. On the basis of all the above data, it seems clear that the days of large ovarian cancer trials open to all patients presenting with the disease have passed.

Origin and Pathogenesis of Ovarian Cancer

Kurman and Shih have considered the origin and pathogenesis of epithelial ovarian cancer and have proposed a unifying theory [12]. They propose that what has traditionally been conceived as primary ovarian cancer actually arises in other pelvic organs, involving the ovary by a secondary process. Endometrioid and clear cell tumors are associated with endometriosis, and they suggest that these may arise in endometrial tissue derived from retrograde menstruation. It is proposed that serous tumors arise from the implantation of benign or malignant epithelium from the fallopian tube. This theory arose from the observation of a tubal carcinoma in a patient with BRCA mutation undergoing risk-reducing surgery. Subsequent systematic and careful examination of the fallopian tube from patients with inherited BRCA mutations undergoing risk-reducing surgery has demonstrated in situ and small early invasive tubal carcinomas in a significant proportion. Further supporting the theory of a fallopian tube origin is the observation that over 70 % of sporadic ovarian and peritoneal high-grade serous carcinomas show mucosal tumor involvement including serous tubal intraepithelial carcinoma, nearly all of which overexpress P53.

Conventional Management of Newly Diagnosed Ovarian Cancer

Conventional management for ovarian cancer for the past 15 years has been primary surgery with the aim of achieving optimal cytoreduction, followed by platinum-based cytotoxic chemotherapy.

Platinum Agents

Cisplatin chemotherapy was introduced in the late 1970s and revolutionized the treatment of ovarian cancer. Carboplatin was introduced in the mid-1980s. Clinical trials demonstrated that carboplatin was as active as cisplatin but was substantially less toxic and in particular could be given as an outpatient without the need for complex pre-and post-hydration regimens. It was also substantially less neurotoxic and less emetogenic [13–17]. In the UK and mainland Europe, single-agent carboplatin became a standard of care, and trials such as ICON2 [18] failed to demonstrate that the three drug combination of cisplatin, epirubicin, and cyclophosphamide was superior to single-agent carboplatin. In the USA, however the standard of care remained the combination of cisplatin and cyclophosphamide.

Paclitaxel with a Platinum Agent

With the advent of paclitaxel, the GOG111 trial demonstrated that the combination of cisplatin and paclitaxel was superior to the previous standard of cisplatin and cyclophosphamide [19]. This was later confirmed by the OV10 trial conducted by the EORTC and the Scottish Gynaecological Oncology Group [20]. The GOG158 trial then demonstrated that the combination of carboplatin and paclitaxel was not inferior to the combination of cisplatin and paclitaxel in terms of response rate, duration of response, or OS, but was considerably less toxic [9]. As a result, the combination of carboplatin and paclitaxel has been the international standard of care for the past 10 years.

The data showing the superiority of platinum and paclitaxel has not however been absolutely consistent, and both the ICON3 trial [21] and the GOG132 trial [22] showed equivalent outcomes for full-dose single-agent platinum treatment when compared to combinations of platinum and paclitaxel. ICON3 compared the combination of carboplatin and paclitaxel chemotherapy to one of two investigator-specified control arms, either full-dose single-agent carboplatin chemotherapy or the combination of cisplatin, doxorubicin, and cyclophosphamide. This trial showed no advantage for the addition of paclitaxel in terms of either PFS or OS [21]. The GOG132 trial compared the combination of cisplatin and paclitaxel to a control arm of full-dose single-agent cisplatin and failed to show an advantage for the combination of cisplatin and paclitaxel [22]. As a result, the UK National Institute for Health and Care Excellence TA55 guidance on the use of paclitaxel in the treatment of ovarian cancer states that "The choice of treatment [between single-agent platinum or a combination of platinum and paclitaxel] for first-line chemotherapy for ovarian cancer should be made after discussion between the responsible clinician and the patient about the risks and benefits of the options available" [23]. However, the US National Comprehensive Cancer Network (NCCCN) Guidelines on epithelial ovarian cancer are less permissive and recommend the use of combination taxane and platinum therapy [24].

Attempts to Improve Outcomes from Modifications to Standard Therapies

Although the introduction of taxanes into ovarian cancer chemotherapy did improve outcome to some extent, the vast majority of patients with suboptimally debulked (>1 cm residuum after primary surgery) advanced disease still developed progressive disease from which they ultimately died with a median PFS and OS in GOG111 of 18 and 38 months, respectively, for patients treated with paclitaxel and cisplatin chemotherapy [19]. Investigators have therefore researched a variety of strategies around optimization of existing therapies and introduction of new drugs into the treatment of this disease, and a number of these strategies are making a significant impact on the outcome of this disease.

Surgical Specialization Issues

Internationally the last 10 years has seen a reorganization of surgical services designed to ensure that clinicians treating ovarian cancer have the appropriate experience and expertise. It has been demonstrated repeatedly that outcomes for patients with ovarian cancer are improved when surgical treatments are performed by those with specialized expertise with the aim of achieving maximal cytoreduction. Paulsen et al. were able to demonstrate that patients having surgery by a specialized gynecologic oncologist had a better outcome in terms of survival than those patients receiving treatment under the care of a general gynecologist or general surgeon. Surgical volume is also important, and those surgeons performing more than ten operations had a substantially improved outcome over those performing between one and ten surgical operations year [25]. An analysis of 3,388 patients enrolled

in three consecutive AGO trials of surgery followed by chemotherapy has demonstrated clearly that those patients debulked to 0 cm of macroscopic residual disease have an outcome that is substantially better than those debulked to between 1 and 10 mm of residual disease and those debulked to more than 10 mm of residual disease, the median survival for these groups of patients being 99.1, 36.2, and 29.6 months, respectively [26]. It has now generally been accepted that optimal surgical debulking means complete macroscopic clearance of disease and no longer reduction to less than 1 cm of residual disease. If surgical treatment is to be performed, then the surgeon needs to be clear that debulking of this sort of extent is feasible. A Cochrane Review of this subject reached the same conclusions [27].

Timing of Surgery

Also under investigation currently is the concept of timing of surgery in ovarian cancer management. Conventionally surgery is used as the initial treatment modality, followed by adjuvant chemotherapy to treat macroscopic or microscopic residual disease. However, many patients are severely symptomatic at the time of presentation with symptoms such as ascites and/or subacute bowel obstruction; they may also have metabolic disturbance such as hypoalbuminemia and renal impairment and may be of relatively poor performance status. It is well known that such patients do badly with initial surgery and there is a high postoperative morbidity and mortality. This has led to the investigation of neoadjuvant chemotherapy given in an attempt to stabilize and improve the patient's condition prior to surgical treatment [28]. This concept has been investigated in the EORTC 55971 trial [29] and also in the UK in the CHORUS trial [30]. Both trials had a non-inferiority trial design. In EORTC 55971, patients with FIGO stages 3C to 4 ovarian carcinoma were randomized to primary debulking surgery followed by platinum- and paclitaxel-based postoperative chemotherapy or alternatively to a neoadjuvant chemotherapy arm, where the same chemotherapy treatment was administered intravenously for 3 cycles of treatment and followed by interval debulking surgery with intent to achieve maximal debulking of disease. This was followed by 3 further cycles of consolidation chemotherapy. Patients could receive chemotherapy with either cisplatin 75 mg meter squared or carboplatin AUC 5. Paclitaxel was administered on a 3-week schedule at 175 mg per meter squared. It was possible to administer docetaxel instead of paclitaxel in the case of paclitaxel allergy. 718 patients were enrolled into the trial. The rate of debulking to $\leq$1 cm of residual disease was 41.6 % at primary debulking surgery and 80.6 % at interval debulking surgery. Primary chemotherapy was non-inferior to primary surgery, but there was no significant improvement in median PFS or median OS, and in a multivariate analysis, assigned treatment arm was not significant in determining overall survival outcome [29].

The CHORUS trial randomized 550 patients, who, on the basis of radiology and serum tumor markers, appeared to have ovarian carcinoma, to receive primary surgery followed by 6 cycles of platinum-based chemotherapy or alternatively 3 further cycles of primary chemotherapy followed by interval debulking surgery followed by 3 cycles of consolidation platinum-based therapy. The results of the CHORUS trial were presented at ASCO 2013 and as yet are only available in abstract form [30]. The patient population was older, of poorer performance status, and with more advanced stage disease than was the case in previously published phase 3 clinical trials in the first-line treatment of ovarian cancer. The median age was 65 (26–88), and 19 % were performance status 2 or 3. Like EORTC 55971, the non-inferiority endpoint was met, but there was no significant improvement in survival outcome for patients receiving primary chemotherapy, although the surgical complication rate was lower [30].

The CHORUS trial is complimentary to the EORTC 55971 trial facilitating a future patient data-level meta-analysis of the two trials. A cross trial meta-analysis presented at ASCO 2013 does suggest a trend to improved overall survival for

neoadjuvant chemotherapy with a hazard ratio of 0.93 (95 % CI: 0.81–1.06) [30].

The ongoing Japanese JCOG 0602 study asks a similar question and compares primary surgery followed by 8 cycles of postoperative carboplatin and paclitaxel with a neoadjuvant approach comprising 4 cycles of the same chemotherapy given prior to interval debulking and 4 afterwards [31].

On the basis of the above data, the role and influence of neoadjuvant chemotherapy have continued to develop, and in many centers, such as our own in Leeds, UK, around 45 % of patients with newly diagnosed ovarian cancer will receive neoadjuvant chemotherapy as their initial treatment. However, there is no universal agreement, particularly from those who espouse ultra-radical surgery, that neoadjuvant chemotherapy is always appropriate, and this subject has been recently reviewed by Vergote et al. [32].

Addition of Alternative or Additional Drugs

There have been attempts to improve on the combination of carboplatin and paclitaxel by the addition of additional cytotoxic drugs normally used in 2nd-line therapy, but the GOG182/ICON5 trial [33] and the OVAR-7 trial [34] and the OVAR-9 trial [35] showed no benefit from the addition of liposomal doxorubicin, gemcitabine, or topotecan to the standard doublet of carboplatin and paclitaxel.

High-Dose Chemotherapy

The role of high-dose chemotherapy with stem cell support has also been investigated but trials failed to recruit adequate numbers due to lack of enthusiasm from patients and clinicians [36]. Trials in other solid tumors such as breast cancer have shown no clear advantage to the high-dose approach which is substantially more toxic. This subject has been reviewed by Ledermann [36] but is not the subject of current investigation in epithelial ovarian carcinoma.

Intraperitoneal Therapy

There is potentially a role for the administration of chemotherapy via the intraperitoneal route. The aim of such treatment is to capitalize on the potential therapeutic advantage derived from exposure of intraperitoneal disease to high local concentrations of cytotoxic drugs, while maintaining systemic absorption of the drugs through the peritoneal membrane. The GOG172 trial [37] enrolled patients with epithelial ovarian cancer with FIGO stage 3 disease debulked to a residuum of 1 cm or less. Patients were randomized to receive conventionally administered intravenous cisplatin and paclitaxel or alternatively intravenous paclitaxel on day 1 with intraperitoneal cisplatin in a dose of 100 mg/m^2 on day 2, with intraperitoneal paclitaxel in a dose of 60 mg/m^2 on day 8.

Only 40 % of patients in the intraperitoneal arm were able to complete 6 cycles of the assigned chemotherapy with full protocol doses, although 83 % of patients were able to complete 6 cycles of some form of chemotherapy. There were significantly more grades 3 and 4 fatigue, pain, leukopenia, GI toxicity, metabolic disturbance, and neurological toxicity with the intraperitoneal administration route. There was however significantly improved progression-free survival and overall survival [37].

The results from this trial were the subject of a clinical alert issued by the National Cancer Institute in the USA [38], which summarized in the form of a cross trial meta-analysis, data from five other trials of intraperitoneal therapy in ovarian cancer. Overall the hazard ratio for survival for the five trials was 0.79 (95 % CI 0.70–0.89); however, despite the improved outcomes for the intraperitoneal route of administration, it has not uniformly captured the enthusiasm of clinicians or patients and has not become an international standard of care in other than a relatively small number of enthusiastic centers.

There are ongoing trials with intraperitoneal chemotherapy running in both Canada and Europe investigating whether the apparent advantage seen in GOG172 [37] might have

come about through the weekly scheduling of paclitaxel on days 1 and 8 of each 3-weekly treatments cycle, rather than necessarily being due to the intraperitoneal approach. Also being investigated is whether the use of carboplatin rather than cisplatin might make this treatment rather more acceptable to patients.

In the UK and in Canada, the PETROC/OV21 trial is recruiting patients who have achieved debulking to 1 cm or less of residual disease at interval debulking surgery [39]. This trial has successfully completed its phase 2 safety phase and is now expanding into phase 3. The trial is expected to complete recruitment towards the end of 2018.

Paclitaxel Scheduling

Other concepts currently under investigation revolve around the scheduling of paclitaxel chemotherapy (referred to above). In 2009, Katsumata et al. [40] published the results of the JGOG 3016 trial where patients with epithelial ovarian carcinoma, primary peritoneal carcinoma, or fallopian tube carcinoma between stages 2 and 4 were randomized to receive conventionally administered carboplatin and paclitaxel (AUC 6 and 180 mg per meter squared, both given on day 1) or alternatively an experimental arm with carboplatin given in the same dose on day 1 with paclitaxel administered in a dose of 80 mg per meter squared on days 1, 8, and 15 of each 3-weekly treatment cycle.

Six hundred and thirty-seven patients were recruited between 2003 and 2005. Although there was no significant change in overall response rates, there was a substantial improvement in median PFS which increased from 17.2 months in the control arm to 28 months in the research arm. In an updated overall survival analysis published in 2013, an OS advantage was confirmed [41]; the median OS for patients in the control arm was 62.6 months but had not yet been reached in the research arm. The hazard ratio was 0.79 with a 5-year survival in the control arm of 51.1 % and in the research arm of 58.7 % (hazard ratio of 0.79; $p = 0.039$). The results of this trial are unprecedented in ovarian cancer research coming about simply by changing the scheduling of one of the two drugs used in the conventional combination chemotherapy regimen.

A further trial (MITO-7), presented at ASCO 2013 and conducted by an Italian Oncology group [42], fractionated both the carboplatin and the paclitaxel, but did not increase the dose intensity of the treatment using paclitaxel at a weekly dose of 60 mgs/m^2 and carboplatin at an AUC of 2.

In comparison with conventionally administered 3-weekly carboplatin and paclitaxel, the experimental arm was significantly less toxic, but there was no improvement in PFS as a result of dose fractionation. Whether this difference in outcome between the trials comes about as a result of the lower-dose intensity employed in MITO-7, the fractionation of the carboplatin dose, or whether it was because it was conducted in a non-Japanese population is unclear.

The GOG have completed recruitment into GOG 0262 [43], a trial designed to replicate the Japanese trial, using paclitaxel 80 mgs/m^2 per week in the research arm with carboplatin AUC 6, but which also allows the use of bevacizumab according to investigators choice in both arms; results from GOG 0262 will be available later this year.

In the UK, the ICON8 trial continues recruitment [44]. Within ICON8, patients receiving either postoperative chemotherapy or neoadjuvant chemotherapy are randomized to three arms, either a conventional carboplatin and paclitaxel control arm or alternatively to the Japanese regimen receiving carboplatin on day 1, with paclitaxel (80 mg/m^2) in a fractionated weekly regimen on days 1, 8, and 15 of each 3-weekly treatments cycle or alternatively to a 2nd experimental arm where both carboplatin and paclitaxel are given on a weekly basis.

It has been suggested that weekly paclitaxel may operate in part via an antiangiogenic mechanism. If this is the case, it will be important to investigate how this approach is best integrated with more conventional anti-VEGF approaches such as bevacizumab. A parallel protocol to ICON8 (ICON8B) is currently being developed in which there will be an additional randomization to bevacizumab. This will facilitate investigation

as to whether the putative antiangiogenic effect of weekly paclitaxel is synergistic with bevacizumab or whether the same effect can be achieved with weekly paclitaxel alone.

Maintenance Chemotherapy

Another concept that has been investigated is the use of conventional chemotherapy drugs as a maintenance agent. A study published by Markman et al. in 2003 [45] randomized 277 patients with stages 3 and 4 epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer, who had achieved remission following 5–6 cycles of adjuvant carboplatin and paclitaxel regimen to receive either 3 further cycles of paclitaxel given in a dose of 175 mg per meter squared over 3 hours every 28 days for 3 cycles or 12 cycles of treatment. The extra 9 months of chemotherapy with paclitaxel resulted in an improvement in median PFS of 7 months, but this was at the cost of significant toxicity with 23 % of patients developing grade 2 or 3 neuropathy when treated with 12 months of paclitaxel. Of the patients, 7.5% had to discontinue treatment for this reason. The trial was stopped early, and, therefore, no comment can be made about the efficacy of this treatment with respect to OS.

A similar study was conducted by the Yorkshire Regional Cancer Organization investigating the role of interferon alpha as a maintenance agent, and again this showed no significant advantage to the addition of interferon alpha [46].

Exploitation of Ovarian Cancer Biology

The past 10 years has seen a revolution in our understanding of ovarian cancer biology. In parallel a range of molecular therapeutics have been developed. There are multiple potential targets for molecular therapy within the family of diseases known as ovarian cancer, and some of the genetic differences between the various histological subtypes and between high- and low-grade tumors have been described above. The three broad areas that can be targeted have been reviewed by Banerjee and Kaye [47] and can be broadly defined into vascular-targeted therapies, agents that inhibit aberrant growth control pathways within tumor cells and agents such as PARP inhibitors that interfere with DNA repair and replication.

PARP Inhibitors

The development of PARP (poly(adenosine diphosphate [ADP] ribose) polymerase) inhibitors stems from the study of the impaired homologous repair mechanisms exhibited by patients with inherited BRCA1 or BRCA2 gene mutation. PARP is responsible for the repair of the DNA single strength breaks that occur on a daily basis, through the repair of base excisions.

If PARP is inhibited, this leads to replication fork collapse, and in the normal healthy situation where patients have normal BRCA gene status, DNA repair proceeds through the intact homologous repair mechanism. However, in patients with BRCA gene mutations, there is impaired homologous repair within the tumor as a result of loss of the remaining wild-type BRCA allele, and alternative error-prone DNA repair mechanisms are invoked leading to chromosomal instability and cell death.

Within the normal cells of the body where homologous repair pathways remain intact, PARP inhibitors are nontoxic leading to the concept of synthetic lethality [48, 49].

Much of the clinical trial work in patients with ovarian cancer has been conducted with the orally administered PARP inhibitor olaparib. Fong et al. [50] conducted an initial proof of principal phase 1 dose-escalation clinical trial which enrolled 60 patients with unselected malignancies, all of whom had received more than 1 previous chemotherapy regimen and 53 % had received 4 or more previous chemotherapy regimens. Twenty-two patients were carriers of a BRCA1 or BRCA2 gene mutation. Treatment with olaparib was well tolerated. Responses were seen only in patients with a BRCA gene mutation and of 19 patients with breast, ovarian, or prostate cancer; durable

and clinically meaningful responses were observed in 12 (63 %).

Subsequent clinical trials with various PARP inhibitors have shown intriguing results. Audeh et al. [51] reported a phase 1 cohort study in 57 patients with recurrent ovarian cancer with a BRCA gene mutation who had previously been treated with a median of 3 previous chemotherapy regimens. Olaparib was administered in a dose of 400 mg twice daily (cohort 1; $n=33$) or 100 mg twice daily (cohort 2; $n=24$). The response rate was 33 % in cohort 1 and 13 % in cohort 2 confirming the efficacy of olaparib in this patient population. In general treatment was well tolerated with nausea and fatigue being the predominant side effect. Grade 1 or 2 nausea occurred in 42 % of patients treated at 400 mg twice daily with grade 3 or 4 in 6 %. Corresponding figures for grades 1 and 2 and grades 3 and 4 fatigue were 30 and 3 %. Similar side effects were seen at the lower dose but occurred in a small proportion of patients. The authors concluded that PARP inhibition has a wide therapeutic window and sufficient tumor cell selectivity to target ovarian cancers with defects in DNA repair by homologous recombination.

The observation from the Cancer Genome Atlas Project that 50 % of patients with high-grade serous ovarian cancer have homologous repair defects has led to the concept of BRCAness, and the hypothesis that PARP inhibitors may be effective in a broader group of patients than just those with BRCA gene mutation.

Gelmon et al. reported a trial of olaparib in a population of patients with high-grade serous or undifferentiated recurrent ovarian carcinoma or triple receptor-negative breast cancer [52]. Patients were stratified according to whether they had a BRCA gene mutation. Of 63 heavily pretreated patients with ovarian cancer (median number of previous treatments = 3), 7 of 17 (41 %) with BRCA gene mutation responded, as did 11 of 46 (24 %) without BRCA gene mutation; all of whom had high-grade serous ovarian cancer. In this trial, the response rate in patients with potentially platinum-sensitive ovarian cancer was 50 % (10/20) in the BRCA-negative cohort and 60 % (3/5) in the BRCA gene mutation cohort. In patients with platinum-resistant ovarian cancer, the response rate was 33 % (4/12) in BRCA gene-mutated patients, but only 4 % (1/26) in those without BRCA gene mutation. This trial confirmed the therapeutic potential of PARP inhibitors in patients with high-grade serous, potentially platinum-sensitive ovarian carcinoma with homologous repair defects occurring through mechanisms other than BRCA gene mutation and led the way to several randomized phase 2/3 studies in patients with earlier stage disease.

Ledermann et al. [53] investigated olaparib as a maintenance agent in 265 patients with recurrent platinum-sensitive high-grade serous ovarian carcinoma who had received 2 or more platinum-based chemotherapy regimens and who had achieved a partial or complete response to their most recent platinum-based regimen. One hundred and thirty-six were assigned to olaparib maintenance in a dose of 400 mg twice daily and 129 to matched placebo. Median PFS was 8.4 months for patients receiving olaparib compared with 4.8 months for those receiving placebo (hazard ratio for progression or death, 0.35, 95 % CI: 0.25–0.49). An interim analysis of OS with 38 % of required events showed no significant difference between the groups (hazard ratio 0.94).

An update of this trial presented at ASCO in 2013 [54] showed the effect to be particularly evident in patients with a germline BRCA gene mutation. The median PFS with olaparib maintenance vs. placebo was 11.2 vs. 4.1 months (HR, 0.17; 95 % CI 0.09–0.32; $p<0.001$). In patients with either tumor or germline mutation of BRCA, a consistent PFS benefit was seen (median: 11.2 vs. 4.3 m; HR, 0.19; 95 % CI 0.11–0.32; $p<0.0001$). The OS analysis showed no significant difference between the arms, but this may have been confounded by the use of post-progression olaparib.

A similar trial from Oza et al. [55] randomized 162 patients with relapsed high-grade serous histology platinum-sensitive advanced ovarian cancer, who had received 3 or less previous platinum-containing chemotherapy regimens to

standard carboplatin and paclitaxel chemotherapy with or without olaparib in a dose of 200 mg twice daily between days 1 and 10 of each 21-day treatment cycle. During the maintenance phase, patients randomized to olaparib continued in a dose of 400 mg twice daily continuously or no further treatment. Patients randomized to olaparib had superior PFS with a median of 12.2 months compared to 9.6 months for those receiving chemotherapy alone (hazard ratio 0.51; 95 % CI: 0.34–0.77). Olaparib was well tolerated in both arms of the trial.

In a subsequent update of the trial presented at the European Cancer Congress 2013, Oza et al. [56] demonstrated that for the 41 patients (38 %) with a known deleterious BRCA gene mutation in either the tumor or the genome, the effect was particularly large (HR 0.21; 95 % CI: 0.08–0.55; $p=0.0015$). Median PFS had not been reached for those treated with olaparib.

On the basis of these data, phase 3 trials have been instigated with both olaparib and rucaparib.

Olaparib is being investigated as maintenance therapy after chemotherapy in patients with a germline BRCA mutation in the first-line setting in the SOLO-1 trial [57] and in the relapsed setting in the SOLO-2 trial [58].

Rucaparib, another PARP inhibitor, is being investigated in the ARIEL2 trial [59] which is a single-arm, open-label study designed to identify tumor characteristics that predict sensitivity to rucaparib using DNA sequencing in relapsed patients with high-grade serous ovarian cancer including those with germline BRCA gene mutation. It is also being investigated in the ARIEL3 trial, a phase 3 randomized study of rucaparib as switch maintenance following platinum-based chemotherapy in patients with platinum-sensitive, high-grade serous or endometrioid epithelial ovarian, primary peritoneal, or fallopian tube cancer [60].

Angiogenesis

Bevacizumab, a monoclonal antibody that binds circulating vascular endothelial growth factor (VEGF), is the most advanced of the molecular therapeutics currently available with 4 large phase 3 clinical trials, 2 in the first-line setting [61, 62], one in platinum-sensitive relapse [63], and a further one in platinum refractory relapse [64]. Bevacizumab is currently licensed by the European Medicines Agency for first-line treatment of advanced ovarian carcinoma and also in the platinum-sensitive relapsed setting.

The process of forming new blood vessels, termed angiogenesis, is a complicated and tightly regulated mechanism involving pro- and antiangiogenic factors. It is a normal adaptive response that is essential for development, reproduction, and repair [65]. In normal physiology, the equilibrium of these factors is shifted to favor the production of proangiogenic factors (e.g., VEGF and Ang2) over antiangiogenic factors (e.g., sVEGFR1, thrombospondins, and semaphorins) resulting in new vessel growth. Proangiogenic factors are regulated by the antiangiogenic factors so that new vessels form an organized network with normal vessels. Once tissue vascularization has occurred, the equilibrium is reestablished to stop new vessel growth. In tumors, abnormal genetic, epigenetic, and metabolic changes are thought to trigger an "angiogenic switch" resulting in constitutive production of proangiogenic factors in excess of antiangiogenic factors [66, 67]. This leads to excessive formation of abnormal blood vessels that are structurally and functionally abnormal [68]. The new vessels and their microenvironment are chaotic and disorganized resulting in abnormal blood flow patterns and increased interstitial fluid pressure that causes leakiness and edema. These abnormal tumor vessels are poorly functioning which not only impairs drug delivery and efficacy but also creates a favorable environment for tumor progression and metastasis [69].

Various factors modulate tumor angiogenesis, none more so than vascular endothelial growth factor (VEGF). Furthermore, abrogation of VEGF-mediated signaling has been consistently shown to have an antitumor effect [70]. VEGF-dependent angiogenesis has been demonstrated in many in vitro and in vivo studies of ovarian cancer [71–74]. VEGF blockade in animal models

was shown to inhibit ascites formation, slow tumor growth [75], and reverse dysfunctional angiogenesis [76].

In patients with ovarian cancer, VEGF was present in ascites [77], and high serum levels were an independent predictor of poor survival [78, 79]. Various strategies have been developed to combat VEGF-mediated signaling. VEGF ligand inhibition with bevacizumab has been extensively studied in ovarian cancer.

Phase II Studies of Bevacizumab

There were several phase 2 studies of bevacizumab monotherapy [80, 81] and bevacizumab in combination with chemotherapy for both first-line and relapsed treatment [82, 83].

The GOG 170D enrolled 62 women with recurrent OC who had received less than two prior chemotherapy regimens and included patients with both platinum-sensitive disease (>6 months platinum-free interval) and platinum-resistant disease(<6 month interval). Forty-eight percent of patients had platinum-resistant disease. Patients were treated with single-agent bevacizumab 15 mg/kg every 21 days. The objective response rate (ORR), median PFS, and median OS were 21 %, 4.7, and 17 months, respectively. Additionally, 40 % of patients remained progression-free at 6 months [80].

Another study utilizing the same dose of single-agent bevacizumab recruited 44 women with platinum-resistant disease who were more heavily pretreated with up to three prior courses of chemotherapy. The ORR, median PFS, and median OS were 16 %, 4.5, and 10.7 months, respectively; approximately, 28 % of patients remained progression-free at 6 months [81]. This study closed early because of three treatment-related deaths; grade 3/4 bevacizumab-related toxicities were as follows: arterial thrombotic events (6.8 %), proteinuria (15.9 %), hypertension (9.1 %), bleeding (2.3 %), wound healing complications (2.3 %), and GI perforations (11.4 %).

Importantly, these two studies demonstrated unique single-agent bevacizumab activity in epithelial ovarian cancer, not evident in other cancers such as non-small cell lung cancer, breast cancer, or colorectal cancer [84].

Bevacizumab was added to carboplatin and paclitaxel in chemo-naïve patients in 2 phase 2 studies [82, 83]. Remarkable response rates of 75 and 80 % were observed, and only 1 of the studies [82, 83] reported 2 cases of GI perforation. Several small phase 2 studies evaluated the addition of bevacizumab to paclitaxel [85], nab-paclitaxel [86], liposomal doxorubicin [87], and topotecan [88] in patients with recurrent ovarian carcinoma. Response rates of 25–63 % were observed, and GI perforation rates were up to 5 %.

Phase 3 studies for both first-line and relapsed treatments were initiated on the basis of compelling data from these phase 2 studies.

Phase III Studies of Bevacizumab

First-Line Treatment

Two large pivotal phase 3 randomized trials, Gynaecologic Oncology Group (GOG) protocol 0218 and International Collaborative Ovarian Neoplasm 7 (ICON7), evaluated the addition of bevacizumab to standard first-line chemotherapy, given concurrently and as maintenance treatment [61, 62]. Both of the trials were conducted in the first-line setting in women with advanced epithelial ovarian cancer, fallopian tube cancer, or primary peritoneal cancer after their primary debulking surgery. ICON7 included women with high-risk early-stage disease (FIGO stage 1/2A, clear cell or grade 3) which was restricted to 10 % of the total study population and inoperable patients so long as debulking surgery was not planned prior to progression. Both trials had similar objectives but differed in several aspects of trial design, patient eligibility, dose, and duration of bevacizumab therapy [61, 62].

GOG-218 was a double-blind, placebo-controlled trial investigating bevacizumab 15 mg/kg in patients with advanced stage 3 (macroscopic optimal and suboptimal) and stage 4 disease [62]. The three treatment groups were chemotherapy alone, chemotherapy plus concurrent bevacizumab, and chemotherapy plus concurrent and maintenance bevacizumab. At the time of primary analysis of PFS, with a median follow-up of 17.4 months, there was a 3.8-month improvement in PFS favoring the chemotherapy

plus concurrent and maintenance bevacizumab group over the chemotherapy alone group (10.3 vs. 14.1 months, HR = 0.72; 95 % CI = 0.63–0.83; $p < 0.001$), which corresponded to a 28 % reduction in risk of disease progression. Progression was defined on the basis of rising CA125 alone as well as RECIST and symptomatic relapse. When the analysis was censored for asymptomatic patients with disease progression defined by CA125 alone, this PFS advantage extended to 6 months (12.0 vs. 18.0 months, HR = 0.645; 95 % CI = 0.551–0.756; $p < 0.001$) [62]. PFS did not statistically differ between the chemotherapy alone group and the chemotherapy plus concurrent bevacizumab group (10.3 vs. 11.2 months, HR = 0.9; 95 % CI = 0.76–1.04; $p = 0.16$). Median OS was not significantly different at 39.3, 38.7, and 39.7 months for the chemotherapy alone, the chemotherapy plus concurrent bevacizumab, and the chemotherapy plus concurrent and maintenance bevacizumab groups, respectively. When the OS analysis was updated in August 2011, median OS was 3.2 months longer in the chemotherapy plus concurrent and maintenance bevacizumab group compared to the chemotherapy alone group, but it failed to reach significance (40.6 vs. 43.8 months, HR = 0.88; 95% CI = 0.75–1.04; $p = 0.0641$). The confounding effect of significant crossover upon progression (40 %) means that the OS data is unlikely to be informative.

ICON7 was an open-label study investigating bevacizumab 7.5 mg/kg (half the dose used in GOG-218) in patients with FIGO stages 2–4 disease including high-risk early-stage disease (FIGO stage 1/2A, clear cell, or grade 3) and inoperable patients [61]. The two treatment groups were chemotherapy alone and chemotherapy plus concurrent and maintenance bevacizumab. At the time of primary analysis of PFS with a median follow-up of 19.4 months, there was a 1.7-month increment in PFS favoring the chemotherapy plus bevacizumab group, corresponding to a 19 % reduction in the risk of disease progression (17.3 vs. 19.0 months, HR = 0.81; 95 % CI-0.70–0.94; $p = 0.004$). The CR and PR rate was significantly higher in the chemotherapy plus bevacizumab group (48 % vs. 67 %; $p < 0.0001$). On further update [89] with a median follow-up of 28 months, the increment in PFS reached 2.4 months (17.4 vs. 19.8 months) [61]. Intriguingly, the PFS curves maximally separated at 12 months, corresponding with the point at which bevacizumab was discontinued, and the curves eventually intersected at 22 months.

There was evidence of nonproportional hazards ($p < 0.0001$), so the restricted mean survival difference (the difference in areas under the whole length of the PFS curves), a more accurate estimate of treatment effect, was estimated [90]. The restricted mean PFS at 42 month was also prolonged by 1.7 months (22.4 vs. 24.1 months, HR = 0.87; 95 % CI = 0.77–0.99; $p = 0.04$) [62]. OS had not been reached. There appeared to be a trend towards improved overall survival with bevacizumab therapy (HR = 0.85; 95 % CI = 0.69–1.04; $p = 0.11$). This survival advantage was especially evident in patients with high risk for progression, the suboptimally debulked stage 3 and stage 4. This subgroup of patients was broadly comparable with the GOG-218 patients (excluding optimally debulked stage III). When predetermined post hoc exploratory analysis was performed on this high-risk group of patients, bevacizumab therapy was associated with a 5.4-month (10.5 vs. 15.9 months, HR 0.68: 95% CI, 0.55–0.85; $p < 0.001$) improvement in PFS and 7.8-month (28.8 vs. 36.6 months, HR = 0.64; 95 % CI = 0.48–0.85; $p = 0.002$) improvement in OS. These results were confirmed in the final OS analysis which was presented as a late breaking abstract at the 2013 European Cancer Congress. The manuscript for this analysis is in preparation, but in the trial as a whole, no improvement in OS was seen. The improvement in both PFS and OS for those at highest risk of recurrence was confirmed, with little benefit seen in macroscopically resected stage 3 patients [91]

Relapsed Treatment

Two trials, OCEANS and AURELIA, investigated bevacizumab therapy in platinum-sensitive and platinum-resistant OC disease, respectively.

OCEANS (Ovarian Cancer Study Comparing Efficacy and Safety of Chemotherapy and Antiangiogenic Therapy in Platinum-Sensitive

Recurrent Disease) was a randomized, double-blind, phase III trial investigating bevacizumab with the combination of gemcitabine and carboplatin(GC) compared with GC plus placebo in 484 women with platinum-sensitive recurrent OC [63]. Patients received 6–10 cycles of GC plus either bevacizumab or placebo which was continued until disease progression or unacceptable toxicity. Bevacizumab therapy was associated with a 4-month increment in PFS (8.4 vs. 12.4 months, HR = 0.484; 95% CI = 0.388–0.605; $p < .0001$), a higher response rate (57 % vs. 78 %; $p < .0001$), and a longer duration of response (7.4 vs. 10.4; HR = 0.534; 95% CI = 0.408–0.698). No OS advantage was achieved (35.2 vs. 33.3 months; HR = 1.027; 95% CI = 0.792–1.33) which could be related to crossover (34.7 %) or the fact that most patients had several further lines of chemotherapy.

AURELIA (Avastin Use in Platinum-Resistant Epithelial Ovarian Cancer) was a phase III trial randomizing 361 patients who progressed within ≤6 months from their platinum-based first-line therapy to receive their physician's choice of second-line single-agent chemotherapy (pegylated liposomal doxorubicin (PLD), paclitaxel, or topotecan) with or without concurrent/maintenance bevacizumab until disease progression [64, 92]. At a median follow-up of approximately 14 months, there was statistically significant and clinically meaningful doubling of PFS (3.4 vs. 6.7 months, HR = 0.48; 95% CI 0.38–0.6; $p < 0.001$) and overall response rate (12.6 % vs. 30.9 %, $p = 0.001$) in the bevacizumab group. The final analysis of OS presented at the European Cancer Congress in 2013 confirmed that while there was no overall improvement in OS, the effect in the weekly paclitaxel arm was substantially greater than in the other chemotherapy arms with a suggestion of a survival advantage for patients receiving treatment with the combination of weekly paclitaxel and bevacizumab [92]. This intriguing observation will need to be confirmed in future trials.

Tolerability, Safety, and QOL Analysis

Bevacizumab in both GOG-218 and ICON7 was generally well tolerated with acceptable toxicities, and there were no new safety concerns. Treatment for bevacizumab-related hypertension was required in 20–25 % of women. The incidence of serious GI adverse events in both trials was less than 3 %. The earlier phase II trials recruited heavily pretreated patients who were at higher risk of serious GI adverse events. GOG-218 identified bowel resection with anastomosis at the time of initial debulking surgery or history of inflammatory bowel disease as independent risk factors for GI perforation [62].

In the OCEANS study of platinum-sensitive recurrent disease [63], grade 3 or higher hypertension (0.4 % vs. 17.4 %) and proteinuria (0.9 % vs. 8.5 %) occurred more frequently with bevacizumab but only led to treatment discontinuation in 3.6 % of patients. There were no GI perforations during treatment. The most common reason for treatment discontinuation was progressive disease at the time of analysis.

In the AURELIA study of platinum-resistant disease [64, 92], the incidence of GI perforation (2 %) and fistula formation (2 %) was very low probably due to the exclusion of patients with more than two previous anticancer treatments, a history of bowel obstruction, abdominal fistula, or clinical evidence of rectosigmoid involvement. Grade 2 or higher hypertension (7 % vs. 20 %) and proteinuria (1 % vs. 11 %) and grade 3 or higher arterial events (0 % vs. 2 %) occurred more frequently with bevacizumab.

In GOG-218, patients receiving chemotherapy plus concurrent bevacizumab reported significantly lower QOL scores compared to those receiving chemotherapy alone during the chemotherapy phase. This difference was not evident in the maintenance bevacizumab phase [93]. In contrast, ICON7 showed that global quality of life improved during the chemotherapy phase for both treatment groups, but the mean global QOL score at the end of maintenance bevacizumab was significantly lower in the bevacizumab group compared to the chemotherapy alone group [61, 94].

Bevacizumab-Related Questions and Future Research

With GOG-218, ICON-7, OCEANS, and AURELIA, we have four positive phase 3 trials

demonstrating the benefits of bevacizumab in OC, but many questions remain.

In the first-line setting, it is unclear whether the PFS (without OS) benefit with bevacizumab will outweigh the toxicity risks, implications on QOL, and financial cost to society. Additionally, we need clarification about the optimal dose and duration of bevacizumab therapy and the optimal chemotherapeutic regimen to apply concurrently with bevacizumab. Given that bevacizumab was beneficial in both first-line and relapsed disease settings, we need to be able to identify the ideal patient and time to utilize bevacizumab.

Cost-Effectiveness and Availability

There have been several attempts to assess the cost-effectiveness of bevacizumab. Cohn et al. (2011) [95] performed a cost-effectiveness analysis using a hypothetical constructed model based on the GOG-218 preliminary results and concluded that bevacizumab was not cost-effective in the first-line setting and the cost-effectiveness of bevacizumab primarily depended on the cost of the drug itself. Another study used the GOG-0178 and GOG-0218 treatment groups to evaluate the cost-effectiveness of consolidation bevacizumab compared to consolidation paclitaxel and concluded that bevacizumab was more costly and less effective than consolidation paclitaxel [96].

A study from Mexico however used the dose and efficacy reported from the ICON7 "high-risk" subgroup of patients (suboptimally debulked stages III and IV) and demonstrated that this could be a cost-effective strategy [97].

The oncology community has been cautious about adopting bevacizumab into clinical practice. Bevacizumab received EU approval for first-line treatment of OC in December 2011 and for platinum-sensitive relapse treatment in November 2012.

The UK National Institute for Health and Care Excellence (NICE) evaluated the cost-effectiveness of bevacizumab for first-line treatment with the licensed 15 mg/kg dose used in GOG-218. The ICER was roughly £144 K per QALY gained [98]. Similarly, NICE evaluated the cost-effectiveness of bevacizumab for platinum-sensitive relapse treatment and estimated an ICER of approximately £149 K per QALY gained for bevacizumab plus carboplatin and gemcitabine [99]. Based on these appraisals, bevacizumab has not been recommended for first-line or platinum-sensitive relapse treatment in the UK. Paradoxically, however, in England, bevacizumab can be accessed in these indications through the National Cancer Drugs Fund [100, 101]. Further evaluation, including that from the ICON7 Health Economics subgroup, is awaited with interest.

The National Comprehensive Cancer Network (NCCN v2.2013) [102] ovarian cancer panel disagreed on the appropriateness of adding of bevacizumab to first-line treatment, assigning it a category three recommendation. The majority of the panel were concerned about the absence of overall survival and/or quality of life benefit and questioned the value of the PFS advantage in light of the toxicities and financial costs. The panel did however support the use of bevacizumab in combination with gemcitabine in platinum-sensitive relapse [102].

Optimal Duration of Therapy

ICON7 and GOG-218 used different durations of bevacizumab therapy (12 and 15 months, respectively). Preclinical trials have shown that sustained VEGF inhibition causes tumor regression [103, 104]. It stands to reason that tumor growth could resume upon stopping VEGF inhibition. Indeed, the ICON7 and GOG-218 PFS survival curves reached maximal separation at the end of maintenance bevacizumab, and the curves eventually intersected when patients were not on treatment. To that end, two trials are investigating the merits of a longer duration of bevacizumab therapy in first-line treatment. RoSiA is a non-comparative study evaluating 36 cycles of bevacizumab therapy [105], and BOOST/AGO-OVAR 17 is randomly assigning patients to 15 (22 cycles) or 30 (38 cycles) months of bevacizumab therapy [106]. The results of these trials are key to understanding optimal use of bevacizumab, and it may be that longer duration bevacizumab is particularly important for those patients at lower risk of recurrence.

The AURELIA and OCEANS trials in recurrent OC used bevacizumab until disease progression, but continuing bevacizumab beyond progression in recurrent OC may be beneficial. This concept is supported by the ML18147 study in metastatic colorectal cancer which showed an OS advantage in patients who continued bevacizumab with second-line chemotherapy compared to those who stopped bevacizumab after first progression [107]. Resistance to chemotherapy and VEGF suppression are thought to be independent processes that may not occur simultaneously, so initial progression does not necessarily imply resistance to VEGF suppression [108]. Two studies will assess the merits of bevacizumab treatment beyond progression. Both MITO 16/MaNGO 2 [109] and GOG-213 [110] randomly assign patients with platinum-sensitive relapse after bevacizumab to receive their second-line chemotherapy with or without bevacizumab.

Optimal Combination

Given the potential advantage of IP chemotherapy and dose-dense chemotherapy over the standard 3-weekly platinum taxane combination in first-line treatment, several trials are exploring the pairing of these strategies with bevacizumab. OCTAVIA [111] and GOG-262 [112] are evaluating the combination of dose-dense paclitaxel and carboplatin with bevacizumab in first-line treatment. Patients in GOG-262 can elect not to receive bevacizumab, so the study will also provide insight into the merits of dose-dense paclitaxel and carboplatin alone. Meanwhile, GOG-252 [113] is evaluating IP platinum and paclitaxel with IV bevacizumab.

Ideal Patient

The cost of bevacizumab therapy is prohibitive and limiting its current availability, but its cost-effectiveness could be improved if its use was restricted to patients who would benefit most from it. The prespecified subgroup analysis in ICON7 showed that the suboptimally debulked stage III and stage IV patients derived the greatest benefit (PFS and OS), perhaps hinting that this is the optimal group of patients to treat with first-line bevacizumab. These patients have a poor prognosis with rapid velocity disease and a short "window of opportunity" for treatment. It makes sense to use the most effective treatment upfront in these patients. Sadly, the GOG-218 study failed to substantiate these findings as its OS analysis was confounded by crossover and future trials are not designed to resolve this question. The AURELIA and OCEANS studies of bevacizumab in recurrent disease also failed to identify a clinical subgroup of patients with a differential advantage.

Since we are not able to predict response based on clinical features, we may have to rely on predictive biomarkers to identify the ideal patient to treat with bevacizumab. Furthermore, if the theory of differential chemotherapy and VEGF resistance and progression holds true, then we need to define vascular disease progression and identify vascular progression biomarkers to determine whether a patient should discontinue or remain on bevacizumab therapy. Whereas, predictive biomarkers have been identified for other targeted therapies such as trastuzumab, predictive biomarkers for bevacizumab have not been identified. So far, translational studies evaluating baseline levels of proangiogenic factors have not produced consistent results.

Optimal Timing of Therapy

In GOG-218, a PFS advantage was demonstrated with concurrent and maintenance bevacizumab and not with concurrent bevacizumab alone, leading some to deduce that the maintenance rather than the concurrent component of bevacizumab was instrumental in delaying disease progression and challenging the need for concurrent bevacizumab. However, GOG-218 did not have a study arm of chemotherapy plus placebo followed by maintenance bevacizumab, so a direct comparison between concurrent and maintenance therapy is not feasible. Moreover, bevacizumab appears to have important synergistic activity with chemotherapy, exemplified by the significant increment in response rates: 67 % vs. 48 %, 78 % vs. 57 %, and 78 % vs. 57 % conferred by concurrent bevacizumab in the ICON 7, OCEANS, and AURELIA studies, respectively. So unless future studies prove otherwise,

bevacizumab should be initiated with chemotherapy to obtain its proven benefit.

As NACT and interval debulking surgery (IDS) become more commonplace in clinical practice, questions about the feasibility and timing of bevacizumab therapy around IDS will be raised. Concerns about perioperative complications related to bevacizumab will have to be formally assessed, and ICON8, GOG-262, and PETROC/OV21 will provide this information.

The lack of OS (despite PFS) advantage in GOG-218 and the OCEANS studies thought to be confounded by crossover may in fact be informative as it suggests that the timing or sequence of bevacizumab administration may not adversely affect overall survival so long as bevacizumab is administered at some stage. The proponents of bevacizumab in first-line treatment would argue that the PFS advantage was greater in first-line treatment (6 months with GOG-218 vs. 4 months with OCEANS) therefore supporting its use before relapse, but this cross-study comparison is not valid and a study randomizing patients to bevacizumab before relapse or after relapse is necessary to answer this question. Obviously, this becomes a moot point if the studies evaluating bevacizumab therapy beyond progression demonstrate a benefit.

Toxicity may influence the timing of bevacizumab therapy. Early phase trials in heavily pretreated patients showed a higher incidence of GI perforation and vascular events compared to subsequent studies with chemotherapy-naive patients. The AURELIA study probably had a low GI perforation rate because it excluded patients with more than two previous anticancer treatments, a history of bowel obstruction or abdominal fistula, or clinical evidence of rectosigmoid involvement [64, 92]. This may support the argument for using bevacizumab early in the course of the disease, but future studies particularly the studies evaluating bevacizumab therapy beyond progression will provide more safety information.

Summary

Four phase III trials have demonstrated the benefits of bevacizumab in first-line treatment and platinum-sensitive and platinum-resistant relapse treatment. However, these benefits have been confined thus far to improving progression-free survival rather than overall survival. Unless overall survival advantage is demonstrated, the extreme cost of bevacizumab will hamper its integration into clinical practice. Furthermore, its use is not without toxicity risks and QOL implications for patients. Many trials are underway to determine the optimal patient, dose, duration, timing, and combination strategy for bevacizumab therapy. With further refinement and with results from translational studies, perhaps bevacizumab will prove to be even more effective in ovarian cancer.

Other Small-Molecule-Targeted Therapies

In addition to the trials of olaparib and bevacizumab referred to above, there have been over the last 12 months a plethora of intriguing and potentially highly relevant new phase 3 clinical trial data presented which have demonstrated a potential role for a range of additional targeted therapies.

Pazopanib (Votrient)

The AGO-OVAR 16 trial is a randomized, double-blind, phase 3 placebo-controlled trial of pazopanib vs. placebo conducted in 940 women who had not progressed after first-line chemotherapy for FIGO stages 2–4 epithelial ovarian, fallopian tube, or primary peritoneal cancer [114]. Placebo or pazopanib, an orally administered multi-targeted tyrosine kinase inhibitor targeting ATP binding sites of VEGFR, PDGFR, and c-Kit receptor which had previously shown activity in a phase 2 trial [115], was administered for a duration of 24 months or until disease progression. Patients treated with pazopanib had an improvement in median PFS of 5.6 months compared to those receiving placebo (17.9 months vs. 12.3 months; HR 0.77; $p = 0.0021$). Survival data is not yet mature with only 35 % of the events required for the definitive analysis. Pazopanib has class-specific toxic effects

including hypertension (G2/3/4 in 52 % vs. 17 %), G3/4 liver-related toxicity in 9 % vs. <1 %, neutropenia in 10 % vs. 2 %, and diarrhea in 8 % vs. 1 %. Other G3/4 toxicities occurring in>1 % included asthenia, thrombocytopenia, plantar-palmar erythrodysesthesia, headache, abdominal pain, proteinuria, and arthralgia. A subsequent analysis of health-related quality of life (HRQoL) [116] demonstrated a small decrement for patients while on treatment and a significant increase in patient-reported diarrhea. Progression and initiation of further chemotherapy also resulted in worse HRQoL; quality-adjusted PFS did however support the net value of maintenance therapy with pazopanib.

Cediranib (Recentin, AZD2171)

Cediranib is an orally administered small-molecule inhibitor of VEGFR2 intracellular signaling. Phase 2 clinical trials demonstrated activity in ovarian cancer [117], and this led to the ICON6 trial, a randomized double-blind placebo-controlled phase 3 trial of cediranib in platinum previously treated patients relapsing more than 6 months after completing first-line chemotherapy, so-called platinum-sensitive ovarian cancer.

All patients received chemotherapy with a platinum-containing regimen and were randomized in a 2:3:3 ratio to a chemotherapy arm with chemotherapy plus placebo followed by placebo during the maintenance phase or to a concurrent arm with chemotherapy plus cediranib followed by maintenance placebo or to a maintenance arm with chemotherapy plus cediranib followed by maintenance cediranib. Cediranib or placebo began at randomization and continued for up to 18 months while the disease remained controlled.

The primary analysis of this trial presented at the European Cancer Congress in 2013 [118] compared the maintenance arm where cediranib was administered during both chemotherapy and maintenance, with the chemotherapy arm where placebo was administered during both treatment phases. Median PFS was increased by 3.4 months from 9.4 to 12.5 months. Hazards were nonproportional, so the restricted mean is the correct statistic and was increased by 3.2 months (HR 0.57; $p=0.0001$). The HR for the concurrent arm was 0.67 with a median PFS of 10.1 months and increase in restricted mean of 2 months when compared with control. Overall survival was a secondary endpoint, and with around 50 % of the patients having died was significantly increased in the maintenance arm when compared with the chemotherapy arm. Again hazards were nonproportional: the median OS increased from 20.3 to 26.3 months; the restricted mean survival time increased by 2.7 months (HR 0.70; $p=0.42$). Toxicity was much as would be anticipated for this class of drugs and included diarrhea, hypertension, and nausea.

In the light of the data from ICON6, AstraZeneca is in the process of rethinking their earlier strategy to discontinue development of cediranib, and further development of this drug in ovarian cancer is to be expected.

Trebananib (AMG 386)

Trebananib is a peptibody that targets angiogenesis through the angiopoietin axis, by binding and neutralizing Ang1 and Ang2 which interact with the Tie2 receptor to facilitate vascular remodeling. Simplistically Ang1 is responsible for vessel quality and Ang2 for vessel quantity. In the TRINOVA-1 trial, presented at the European Cancer Congress in 2013 [119], 919 patients with recurrent ovarian cancer who had received 3 or less prior chemotherapy regimens, whom had a progression-free interval of <12 months, and who had evaluable or measurable disease were randomized in a 1:1 ratio to receive chemotherapy with weekly paclitaxel plus placebo or weekly paclitaxel plus trebananib given intravenously also on a weekly basis. Chemotherapy and trebananib/placebo continued until disease progression or unacceptable toxicity. The primary endpoint was PFS, which was significantly improved from 5.4 to 7.2 months (HR 0.66; $p<0.001$). Overall response rate as assessed by RECIST criteria was also significantly improved from 29.8 to 38.4 % for patients treated with trebananib. An interim analysis of OS with only 50 % of the events required for the final analysis showed a nonsignificant trend in favor of trebananib (HR 0.86; $p=0.19$).

In comparison with other antiangiogenesis treatments, trebananib was well tolerated with

low levels of hypertension and proteinuria; it did however cause a vascular leak syndrome characterized by edema, pleural effusion, and ascites occurring more frequently in women treated with trebananib.

There are other ongoing trials with trebananib in ovarian cancer; TRINOVA-2 investigates the addition of trebananib to pegylated liposomal doxorubicin in patients with partially platinum-sensitive or platinum-resistant recurrent ovarian carcinoma [120]. TRINOVA-3 is a first-line study investigating the role of trebananib in combination with standard carboplatin and paclitaxel chemotherapy [121]. The results of these trials are awaited with interest.

Nintedanib (BIBF 1120, Vargatef)

Nintedanib is an orally administered triple kinase inhibitor. It is a potent inhibitor of vascular endothelial growth factor receptor (VEGFR) as well as platelet-derived growth factor receptor (PDGFR) and fibroblast growth factor receptor (FGFR).

A randomized phase 2 trial of nintedanib as a maintenance agent in 83 patients with relapsed ovarian cancer who had responded to prior chemotherapy demonstrated a close to significant improvement in 36 week PFS (16.3 % vs. 5 %; HR 0.65; $p=0.06$) [53].

This encouraging result led to the GCIG/ENGOT Intergroup AGO-OVAR 12 trial, the results of which were presented at the European Society of Gynaecological Oncology meeting in 2013 [122]. This was a trial conducted in the 1st-line setting in 1,366 patients with FIGO stages 2B–4 epithelial ovarian cancer, who had been treated with primary surgery. Eligible patients were randomized in a 2:1 ratio to receive carboplatin and paclitaxel in standard doses with either nintedanib or a matched placebo. Nintedanib/placebo was administered in a dose of 200 mg twice daily. The primary endpoint, PFS, was significantly longer with nintedanib than with placebo (17.3 vs. 16.6 months; HR 0.84; $p=0.0239$). Interestingly and contrary to experience with bevacizumab, the benefit appeared to be greater in those patients at lower risk of progression (FIGO stage III disease with residual disease of less than or equal to 1 cm or FIGO stage II disease). In this group median PFS for patients receiving nintedanib was 27.1 months compared with 20.8 months for those treated with placebo (HR 0.75; $p=0.005$). OS data are immature. The predominant toxicities of nintedanib (grades 3–5) were diarrhea (22 % vs. 2 %), hepatic toxicity (16 % vs. 3 %), thrombocytopenia (18 % vs. 6 %), anemia (14 % vs. 7 %), and neutropenia (42 % vs. 36 %). Further follow-up of this trial is keenly awaited, particularly the data with respect to OS. It is clear that further investigation of predictive factors will be required.

Other randomized phase 2 and 3 studies ongoing, or planned, with nintedanib in advanced or recurrent ovarian cancer include the Metro BIBF study [123] where nintedanib is investigated in a placebo-controlled randomized phase 3 trial in combination with metronomic cyclophosphamide. Nintedanib is also being investigated in a randomized phase 2 neoadjuvant study in newly diagnosed patients with FIGO stages 3C–4 disease who are planned for neoadjuvant chemotherapy and maximum effort surgical cytoreduction conducted as an interval debulking procedure [124]. There has been concern about the use of bevacizumab in this group of patients because of its potential effects on wound healing. Nintedanib is therefore a potential alternative. Comparative trials with bevacizumab may well be required.

Management of Specific Subtypes of Ovarian Cancer

As our understanding of the morphological and molecular differences of ovarian cancer develops, it becomes appropriate to begin to consider whether different subtypes of ovarian cancer should be treated in distinct ways.

Low-Grade Ovarian Carcinoma

The biology and management of low-grade serous ovarian carcinoma have recently been reviewed by Romero et al. [125]. Low-grade serous ovarian carcinoma represents 8–10 % of cases of ovarian carcinoma. It tends to occur at a

young age and responds poorly to conventional cytotoxic chemotherapy, but in comparison with high-grade ovarian carcinoma has relatively prolonged overall survival. Low-grade serous ovarian carcinomas are closely related to serous tumors of low malignant potential and may have a common etiology. Some 60 % of low-grade serous carcinomas are associated with serous tumors of low malignant potential, whereas this is the case for only 2 % of high-grade serous carcinomas [126]. When serous tumors of low malignant potential relapse, they often do so in the form of low-grade serous carcinoma.

It has long been recognized that low-grade serous carcinoma responds poorly to conventional platinum-based chemotherapy; however, currently this still remains standard first-line adjuvant or palliative therapy.

To date the only non-chemotherapeutic treatment available outside the context of clinical trials has been endocrine therapy. Given that in breast cancer there is an association between hormone receptor status and response to endocrine therapy and also an inverse association between histological grade and hormone receptor positivity, it might be expected that low-grade ovarian cancer would respond preferentially to endocrine therapy. However, this association remains the subject of ongoing investigation. Wong et al. have provided some supportive data, demonstrating ER and PR positivity in 58 and 43 % of 44 low-grade ovarian carcinomas and in only 27 and 17 % of 48 high-grade tumors, respectively [127].

There are a number of published studies of endocrine therapy in ovarian carcinoma using drugs such as tamoxifen [128], aromatase inhibitors [129], and more modern endocrine agents such as fulvestrant [130]. A Cochrane Review of tamoxifen found 32 articles describing the use of tamoxifen in relapsed ovarian carcinoma, but no randomized studies were identified [131]. The vast majority of the trials of endocrine therapy have been conducted in unselected patients with all subtypes of ovarian carcinoma who were resistant to conventional therapies.

Conventionally clinical benefit from endocrine therapy (in metastatic breast cancer) is defined as those patients who achieve complete or partial response together with those who achieve stable disease for a period of at least 6 months. The trials of various endocrine agents in unselected patients with recurrent ovarian cancer have been reviewed by Argenta et al. and demonstrated clinical benefit in around 1–25 % of unselected patients [132].

Bowman et al. [133] demonstrated stable disease for at least 12 weeks in 10 of 50 patients treated with letrozole but no UICC complete or partial responses. However, when response was determined according to Ca125 levels, 5 of 54 patients achieved at least a 50 % reduction in levels, and a further 14 patients achieved stable marker levels. Exploratory analysis suggested that both UICC and Ca125 responses were associated with ER positivity. A subsequent study from the same group [129] in 42 patients with ER positive previously treated ovarian cancer progressing according to Ca125 criteria showed a Ca125 response in 7 (17 %) and stable disease for at least 6 months in a further 11 (26 %). In 33 patients evaluable for radiological response according to UICC criteria, 3 (9 %) had a PR, and a further 14 (42 %) had stable disease at 12 weeks. Response rates according to Ca125 criteria increased with increasing ER immunoscore levels.

A subsequent trial restricted to ER-positive ovarian cancer with a median of 5 previous treatments used the pure antiestrogen drug fulvestrant [130] and demonstrated a Ca125 response rate of 8 % (1 CR and 1 PR in 26 evaluable patients); 9 (35 %) achieved stable disease. By RECIST response criteria, 13 (50 %) achieved stable disease. A subsequent translational analysis [132] showed that response was associated with levels of ER alpha and was also associated with the ER-regulated marker vimentin. PFS was associated with both vimentin and TIFF1, another estrogen-regulated marker.

There is very little data for the use of endocrine therapy specifically in patients with low-grade ovarian carcinoma. Gershenson et al. [134] identified 64 patients treated with 89 separate hormonal treatment programs over a 20-year period. The overall response rate was 9 % (6 CR and 2 PR); 61 % achieved a progression-free

survival of at least 6 months. Median time to progression was 8.9 months for patients with ER-/PR-positive disease and 6.2 months for those with ER-positive/PR-negative disease.

Another retrospective analysis of 194 patients treated between 1977 and 2009 with hormonal consolidation treatment showed a trend toward improved progression-free survival for those who received hormonal maintenance therapy after completion of conventional chemotherapy management [135].

Perhaps the most promising novel approach for the management of patients with low-grade ovarian carcinoma comes from exploitation of the molecular aberrations that characterize the disease.

As previously stated, the MAP kinase pathway is activated in around 80 % of low-grade serous carcinomas. In a recently published phase 2 study, the MEK inhibitor selumetinib was studied in 52 patients with recurrent low-grade serous ovarian or peritoneal cancer [136]. Selumetinib was administered orally twice daily until progression. The objective response rate was 15 % (8/52), with one patient achieving CR, 7 PR, and 34 (65 %) achieved stable disease. Median PFS was 11 months; median OS has not been reached. The treatment was generally well tolerated. The predominant toxicities were gastrointestinal (grade 3 in 25 %), dermatological (grade 3 in 17 %), and metabolic (grade 3 in 13 %). Twelve percent of patients experienced grade 3 fatigue, 8 % grade 3 pain, and 8 % grade 3 anemia. There was a low level (less than 5 %) of neutropenia and cardiac, constitutional, hemorrhagic, and pulmonary toxicity. This study clearly demonstrates the potential efficacy of this approach in low-grade serous ovarian carcinoma.

An international phase 3 trial (the LOGS trial) which will be conducted in women with low-grade ovarian carcinoma that has recurred or progressed following first-line platinum-based chemotherapy and will compare another MEK inhibitor trametinib with investigators' choice of weekly paclitaxel, liposomal doxorubicin, weekly topotecan, letrozole, or tamoxifen is in the advanced stage of planning and should open to recruitment in early 2014.

Studies with an alternative MEK inhibitor, pimasertib, alone or in combination with SAR245409 (a selective oral inhibitor of PI3K and mTOR) is also underway [137].

An essential component of the new generation of studies with targeted molecular agents is inbuilt translational studies incorporating paired biopsies. These trials will facilitate studies to demonstrate the efficacy of the drug in hitting its molecular target and the influence of measurable intra- and extracellular events on outcome, which ultimately will help determine those patients and cancers that stand to gain the most benefit from the treatment under study.

Clear Cell Ovarian Carcinoma

As previously stated, clear cell ovarian carcinoma is being increasingly recognized as a distinct subtype of ovarian carcinoma with specific morphology and molecular phenotype. The Japanese Gynaecologic Oncology Group has so far lead the way in clinical research specifically directed at clear cell ovarian carcinoma. They carried out an initial phase II clinical trial in 99 patients which compared conventional chemotherapy with carboplatin and paclitaxel with an experimental chemotherapy regimen of cisplatin and irinotecan. Although there was no overall difference in outcome between the 2 chemotherapy regimens, there was a nonsignificant trend towards improved progression-free survival for those patients surgically cytoreduced to less than 2 cm of residual disease who received chemotherapy with cisplatin and topotecan [138]. This intriguing initial result has led to the development of an international phase 3 clinical trial investigating the same combination in a larger group of patients. This trial has now completed recruitment, and the results are awaited [139].

The specific molecular abnormalities that characterize clear cell ovarian carcinoma and the potential for exploitation of these have formed the basis of several recent reviews [7, 140–142]. With the increasing development of specific targeted molecular therapies, there is the opportunity to exploit the specific molecular

characteristics of this disease. As yet, there are no results from mature trials carried out specifically in clear cell carcinoma, but several clinical trials are ongoing or planned.

In an attempt to capitalize on the proangiogenic effects of the IL6-STAT-HIF upregulation commonly seen in clear cell carcinoma, the Gynaecologic Oncology Group is conducting a clinical trial with sunitinib (a small-molecule multitargeted receptor tyrosine kinase inhibitor which inhibits both PDGFR and the VEGF receptor signaling) in recurrent clear cell carcinoma (GOG 254 [143]).

In the UK a randomized phase II clinical trial is planned in patients with relapsed clear cell carcinoma, where patients will be randomized between the multitargeted receptor tyrosine kinase inhibitor nintedanib (referred to above) and chemotherapy of physician's choice chosen from weekly paclitaxel, pegylated liposomal doxorubicin, or topotecan administered on a weekly basis.

The Gynaecologic Oncology Group is also investigating the potential of modulating the PI3 kinase/AKT/MTOR pathway activation brought about through PTEN loss, PIK3CA mutation, or AKT2 amplification. GOG 268 [144] is a phase II trial studying the combination of paclitaxel, carboplatin, and temsirolimus followed by temsirolimus maintenance for an additional 11 cycles, in patients with newly diagnosed stages 3 and 4 clear cell ovarian carcinoma.

Mucinous Ovarian Carcinoma

The subject of mucinous ovarian cancer has recently been reviewed by our group [145]. With the increasing expertise and specialization of gynecological radiologists and pathologists, mucinous ovarian carcinoma is becoming an increasingly rare diagnosis. A key distinction for pathologists is to distinguish between primary mucinous ovarian carcinoma and metastatic involvement of the ovary from primary disease arising in the upper or lower gastrointestinal tract or pancreas. For the patient, this is a key distinction since patients with stage I mucinous ovarian cancer may well have an excellent prognosis [146], whereas those with metastatic disease involving the ovary are likely to have a prognosis measured in terms of months rather than years. Patients with advanced stage primary mucinous ovarian cancers are generally accepted to have a poor prognosis [2] and to respond poorly to platinum- and taxane-based chemotherapy [147]. A recent study of the molecular classification of mucinous ovarian cancer has described KRAS mutation in 44 % of 189 mucinous carcinomas and HER2 amplification or overexpression in 19 % of tumors. Only 4 of 71 mucinous carcinomas where both HER2 and KRAS were known co-expressed both molecular markers. Sixty-six percent expressed either HER2 amplification or KRAS mutation, and 33 % expressed neither.

Historically mucinous carcinoma has been included together with the more common subtypes of the disease in nonselected randomized phase 3 trials. However, the poor prognosis of this histological subtype and its poor response to chemotherapy have led the ovarian cancer community to question this strategy, and the GCIG in its 2010 consensus statement recommended that specific clinical trials should be developed for the rare subtypes of the disease (clear cell carcinoma, mucinous carcinoma, and low-grade serous carcinoma) [148].

The morphological similarities between mucinous carcinoma of the ovary and mucinous carcinomas of the GI tract led researchers to investigate chemotherapy regimens normally used in GI cancer. Phase 2 studies demonstrated that oxaliplatin and 5FU had activity and an acceptable toxicity profile in platinum-pretreated relapsed ovarian cancer in general [149, 150]. In vitro studies conducted in mucinous ovarian cancer cell lines have shown activity of oxaliplatin and 5FU in cisplatin-resistant experimental models [151].

A phase 2 study demonstrated activity of irinotecan in combination with mitomycin-C in 25 patients with platinum refractory mucinous ovarian carcinoma [152] with a response rate of 52 % and median overall survival of 15.3 months. These data informed the design for the mEOC trial, an international phase 3 randomized clinical trial run through the GCIG and designed

specifically for patients with primary mucinous ovarian cancer. mEOC was designed to compare standard chemotherapy with carboplatin and paclitaxel plus or minus bevacizumab with the novel combination of capecitabine and oxaliplatin plus or minus bevacizumab. Unfortunately due to the increasing rarity of mucinous ovarian cancer, the mEOC trial recruited only 49 of the planned 332 patients between 2010 and 2013 and as a result was closed to recruitment in 2013.

Future trials for this uncommon subtype of ovarian cancer will require more determined international cooperation, possibly from the entire GCIG, and are likely to investigate targeted molecular therapy directed to exploit the HER2 amplification or KRAS mutation seen in 66 % of mucinous ovarian carcinomas. There is very preliminary data with trastuzumab in HER2-positive mucinous ovarian carcinoma, but it is too limited to draw any conclusions [153]. Cetuximab has clear activity in colorectal carcinoma, but its activity is restricted to those without mutated KRAS. Cell line data has shown that cetuximab inhibits the growth of cell lines without RAS gene mutations in vitro [154], and clinical studies would clearly be required to investigate this hypothesis further. Targeting mutated RAS is challenging, and this subject has been recently reviewed [155]; studies of mucinous ovarian carcinoma with mutated KRAS will need careful design, incorporating translational science, which should also be incorporated into all clinical trials in ovarian cancer, but particularly in the rare subtypes.

Conclusion

As we enter 2014, the field of ovarian cancer research has never been more interesting. Our understanding of the origins of the disease and its molecular phenotype has been revolutionized by advances in histology and molecular biology. We have available to us the first of a generation of licensed molecular therapeutics in the form of bevacizumab. A range of other molecularly targeted agents have been shown to have activity in the disease, and the exact place of these drugs in molecular-guided therapy remains to be determined, as does their role in combination therapy with chemotherapy or indeed with each other.

We now understand that the disease that we know anatomically as ovarian cancer is made up of a series of distinct molecular subtypes with very different drivers and therefore potentially requiring treatment geared to the distinct molecular phenotype of the patient's individual tumor. The past 20 years of ovarian cancer research has been geared to the large phase 3 clinical trial requiring international cooperation to complete and often recruiting from the whole population of patients requiring chemotherapy for ovarian cancer. We are already seeing specific clinical trials developing for specific subtypes of disease such as clear cell, or mucinous carcinoma, and also for low-grade or indeed high-grade serous carcinoma.

As new clinical trials are developed, it is clear that high-quality translational research has to be central to the trial design. As molecular therapeutics become ever more commonplace, the drugs bill seems set to increase exponentially unless we can determine in advance which patients are destined to respond to the specific therapeutic in question. Unless we can avoid treating patients with no chance of benefit from highly expensive targeted agents, they will never become cost-effective and available for general use. Measurement of quality of life and health economics will be key to patient and regulatory authority acceptability.

The histopathologist however remains a central member of the core multidisciplinary team charged with treating ovarian carcinoma. Clinicians rely on the pathologist to provide an anatomical diagnosis, to determine the pathological stage and histological grade, to distinguish between primary and secondary involvement of the ovary, and to provide standard molecular diagnostics such as hormone receptor status or Her2 where these can be done via immunohistochemistry or in situ hybridization. As molecular profiling becomes even more commonplace, histopathologists may need to extend their remit and skill set, or alternatively molecular pathologists may need to join the MDT.

As a result of the refinement and development of the advances described in this chapter, we are convinced that the outcome for patients with the disease known as ovarian carcinoma will be revolutionized in the years to come.

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5 Integration of Imaging and Pathology in the Multidisciplinary Process

John A. Spencer, Michael J. Weston, and Nafisa Wilkinson

Abstract

Ovarian cancer is the leading cause of death from genital tract cancer in women, and this results from the advanced stage of disease at which the majority of women present in clinical practice. At presentation, most women have metastatic disease in the peritoneal and/or the pleural cavities. The diagnostic methods need to determine that the disease results from ovarian cancer rather than a variety of other malignancies which can present in this way.

Introduction

Ovarian cancer is the leading cause of death from genital tract cancer in women, and this results from the advanced stage of disease at which the majority of women present in clinical practice. At presentation, most women have metastatic disease in the peritoneal and/or the pleural cavities. The diagnostic methods need to determine that the disease results from ovarian cancer rather than a variety of other malignancies which can present in this way.

Advanced ovarian cancer is often termed the "silent killer." Its symptoms are vague and nonspecific or mimic those of other conditions. Hence, by the time a woman with ovarian cancer meets a gynecologist, she may have been investigated by a variety of doctors in primary and secondary care for abdominal symptoms. The most common imaging tests requested initially are abdominopelvic ultrasound (US) or computed tomography (CT).

A minority of women with ovarian cancer have disease confined to the ovary at presentation. These women have a complex adnexal mass which may produce a variety of pelvic symptoms. The diagnostic methods here need to distinguish ovarian cancer from the other complex masses which can develop in the adnexa. Pelvic

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US is the most common initial imaging test and whenever possible should include interrogation with transvaginal US (TVUS). However, many complex adnexal masses are actually the result of benign disease and when US cannot make this distinction MR imaging can help.

The aims of imaging are firstly to establish as confidently as possible that ovarian cancer is the diagnosis and secondly to determine the extent of any metastatic disease. These factors influence the choice of primary management: simple surgical resection, radical debulking (cytoreductive) surgery, neoadjuvant chemotherapy with the intention of subsequent interval debulking surgery (IDS), or palliative care. Unlike the other female genital tract cancers where a tissue diagnosis prompts imaging assessment, this is less commonly obtained before surgery with ovarian cancer. Surgery needs to be appropriate to the diagnosis and to be performed by a gynecologic surgeon with appropriate skill. Hence, diagnostic methods define not only what therapy should take place but also where it should be delivered. Decisions are made in discussion at a weekly multidisciplinary team meeting (MDTM).

When there is extensive peritoneal tumor or when the woman is unfit for radical primary surgery, it is now usual to offer primary (neoadjuvant) chemotherapy. To guide this therapy, a tissue diagnosis is preferred and this can be obtained in most cases using image-guided core biopsy (IGCB).

Thus the goals of diagnostic tests for suspected ovarian cancer are:

- That all elective ovarian cancer surgery is performed by specialist surgeons
- That women with "inoperable" disease are identified (Fig. 5.1)
- That malignancy metastatic to the ovaries is recognized (Fig. 5.2)
- That women with benign complex masses do not undergo cancer surgery (Fig. 5.3)

There is now a strong evidence base which informs the choice and order of diagnostic tests in the diagnosis of suspected ovarian cancer. Key among these are magnetic resonance (MR) imaging for interrogation of sonographically indeterminate complex adnexal masses and imaged-guided core biopsy (IGCB) of peritoneal, omental, or pelvic masses to obtain a tissue diagnosis. These tests fit within a diagnostic pathway (Fig. 5.4) [1].

Learning Points: Introduction to Diagnostic Methods

- Most women with ovarian cancer present at advanced stage.
- US is the first line investigation of suspected ovarian cancer.
- CT is used for the assessment of the extent of tumor spread and look for other primary tumors which may be mimics, e.g., within the colon.
- Image-guided core biopsy (IGCB) provides a tissue diagnosis for peritoneal carcinomatosis when surgery is not planned.
- A minority of women present with a complex adnexal mass without abdominal spread.
- MR is valuable to distinguish complex benign from malignant masses.

Ultrasound at Diagnosis

Most ovarian lesions found on gynecological investigation are benign. The ovaries during menstrual years go through physiological changes that may produce a variety of cysts, hemorrhagic lesions, and corpus lutea. Postmenopausal women also frequently exhibit simple benign ovarian cysts of no consequence. The challenge for ultrasound is to identify those lesions that need further investigation and follow-up without unduly worrying women with normal physiological processes.

It is important to know:

- The patient's age and menstrual status. Adnexal masses in premenarchal or postmenopausal women are more likely to be malignant.
- The date of the first day of the last menstrual period and the normal length of the cycle.

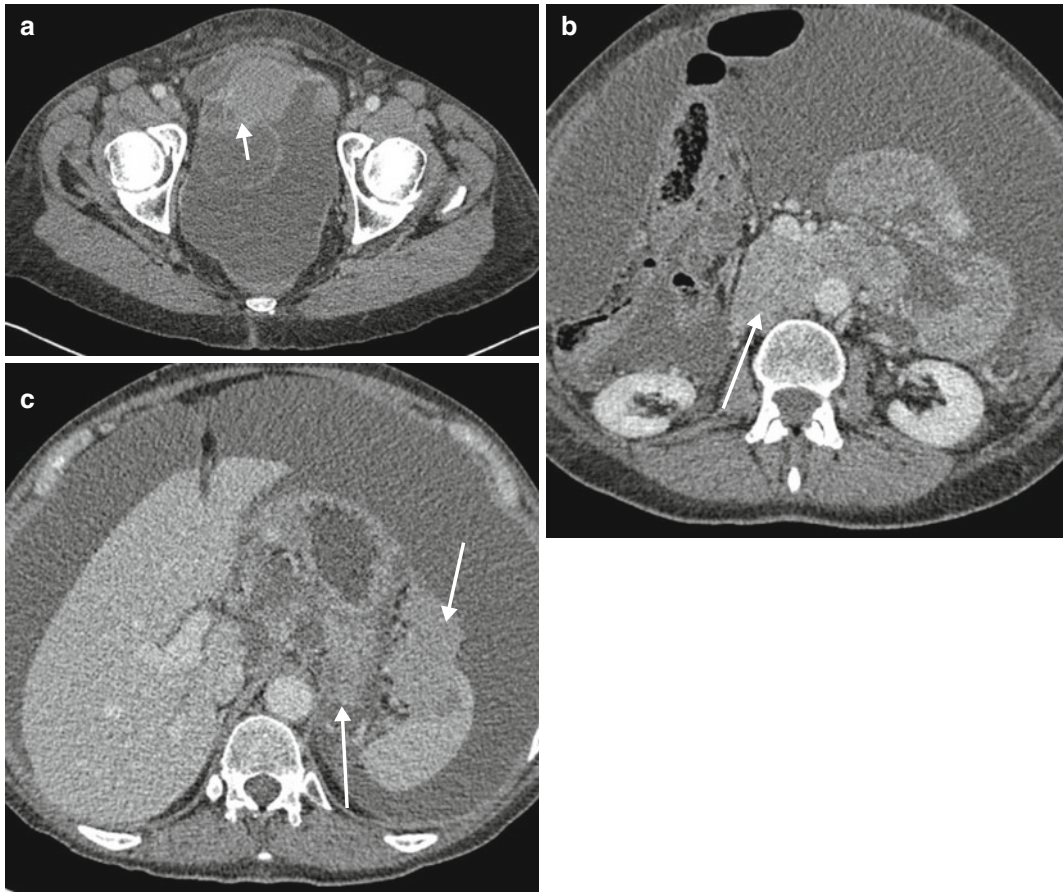

Fig. 5.1 A woman presenting with a CT pattern of "unresectable" ovarian cancer showing (**a**) a malignant right ovarian tumour containing a solid nodule (*arrow*) and sites of "unresectable" disease in (**b**) retroperitoneal nodes (*arrow*) and (**c**) the gastrohepatic ligament and invading the spleen (*arrows*)

- Any medications such as hormone replacement therapy, contraceptives, or tamoxifen.
- Her history of prior gynecological procedures and any comorbidities.

An ultrasound examination should ideally comprise both transabdominal and transvaginal approaches. The transabdominal route gives a larger field of view and is good for assessing large masses and for checking the upper abdomen but uses a lower-frequency probe that has poorer resolution. The transvaginal route has a relatively restricted field of view but has a higher frequency probe that allows better resolution and detailed views of pelvic structures. It can also be used to assess the "visceral slide" of adjacent pelvic structures.

Ultrasound Signs Used to Distinguish Benign from Malignant Cysts

Size

Lesions over 5 cm in diameter are more likely to be malignant. However, very large cysts of 20 cm or more without any signs of disease outside of the cyst are more likely to be benign. Size alone is not a good discriminator.

Calcification

Calcification in otherwise normal ovaries is of no concern but if associated with other abnormal features raises the index of suspicion. These need

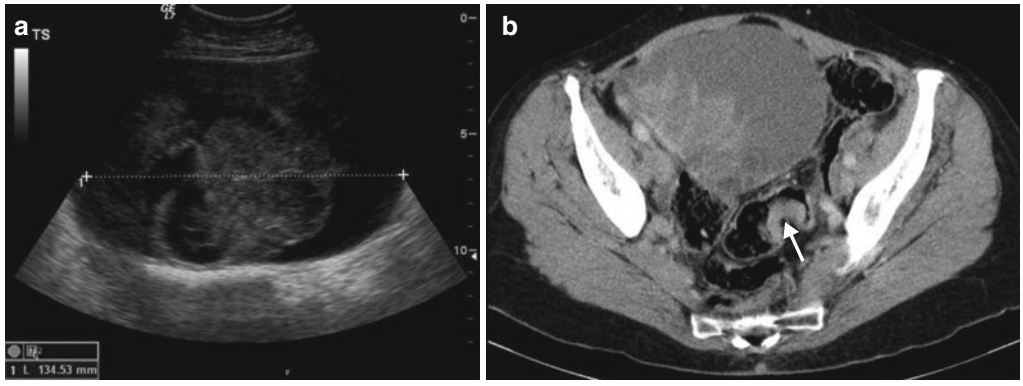

Fig. 5.2 (**a**) Transabdominal US showing a large cystic solid ovarian mass in keeping with malignancy and (**b**) contrast enhanced CT of the pelvis revealing a small non-obstructing tumour of the sigmoid colon (*arrow*). Surgical pathology showed the ovarian mass to be a metastasis from the colon

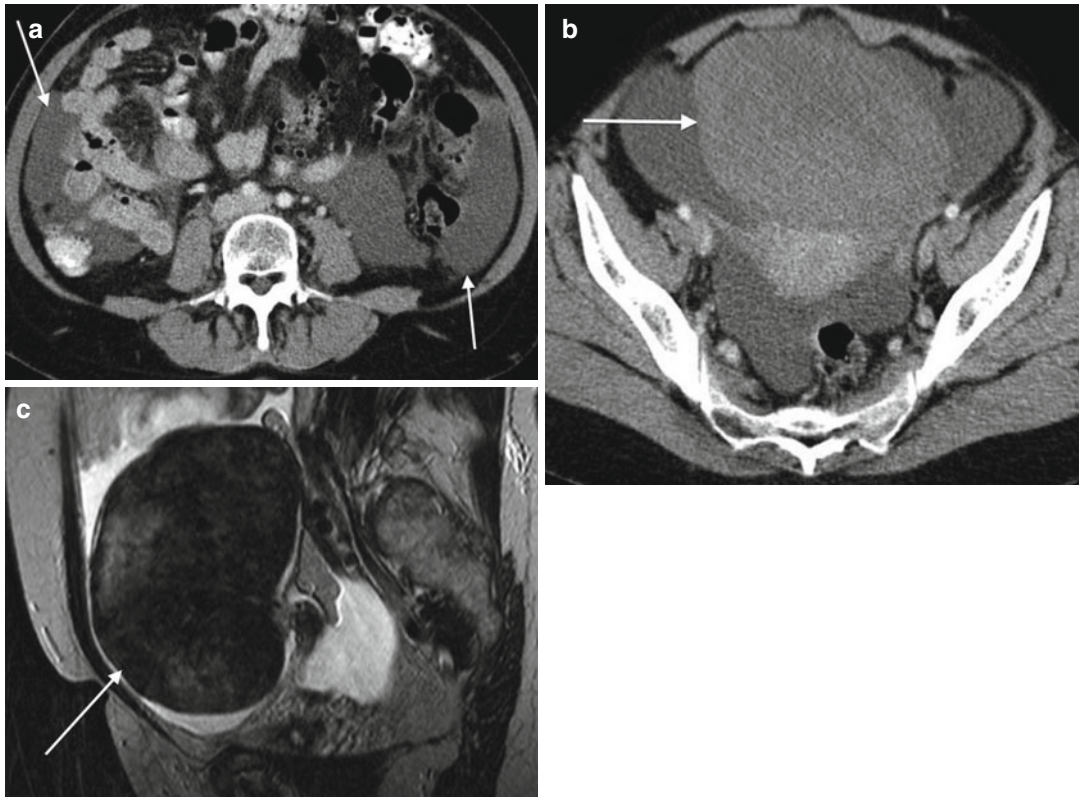

Fig. 5.3 (**a**) Contrast enhanced CT of the abdomen showing ascites (*arrows*) and (**b**) of the pelvis showing a large solid ovarian mass (*arrow*) then (**c**) sagittal plane T2-weighted MR imaging showing a dark solid mass (*arrow*) with features of an ovarian fibroma. Surgery confirmed the diagnosis of Meig's syndrome

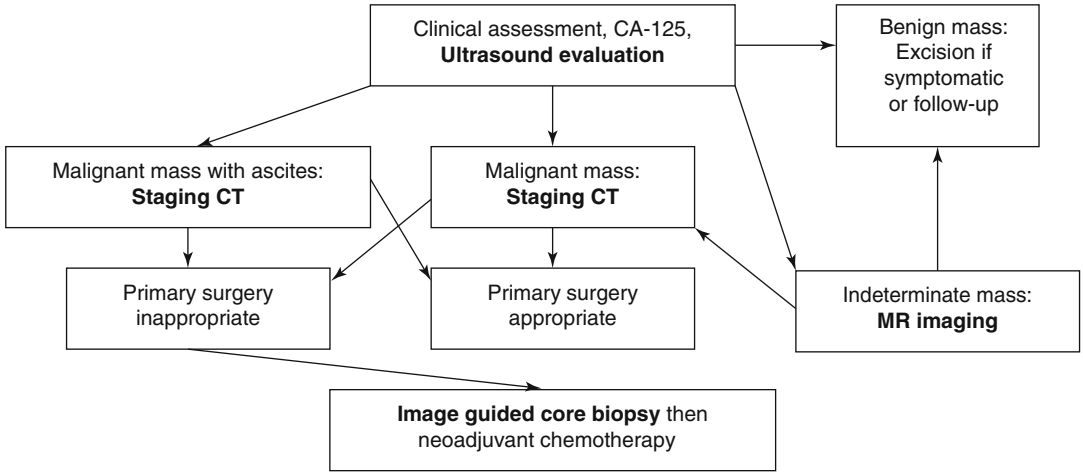

Fig. 5.4 Imaging pathway for suspected ovarian cancer

to be distinguished from the well-recognized features of shadowing echogenic plugs in benign teratomas. Endometriomas also exhibit punctate marginal calcifications.

Septa

Thin septa of less than 3 mm are unlikely to indicate malignancy even when multiple. Septa of over 3 mm thick with an irregular lumpy profile are pointers toward malignancy.

Wall Thickness

An irregular thickened wall of over 3 mm is a pointer toward malignancy, but care needs to be taken not to misinterpret remaining normal ovarian tissue around the margin of a cyst for an area of wall thickening

Papillarities

Nodules or mural vegetations of over 3 mm in size protruding into the lumen of a cyst are a strong marker for malignancy.

Mixed Cystic and Solid Masses

A truly mixed cystic and solid mass is almost pathognomonic of malignancy though cystic adenofibromas can cause confusion.

Blood Flow

The presence of detectable blood flow on color or power Doppler within0 the solid components of a mass increases the likelihood of malignancy.

Contrast Enhancement

Microbubble agents can be used to demonstrate the enhancement of solid components of a mass on ultrasound. This is a strong pointer toward malignancy.

Abnormalities Outside the Lesion of Concern

Ascites is a strong pointer to peritoneal dissemination but care has to be taken not to confuse the small amount of fluid that is commonly seen in the pelvis of women of menstrual years. Benign lesions such as fibromas, torsion, or infection can also produce ascites.

Finding omental cake, peritoneal thickening, or lymphadenopathy is highly indicative of advanced malignancy.

Confounding Features

Hemorrhage into a lesion and subsequent clot retraction can mimic the presence of fat or solid tissue. Follow-up scans will show the clot resolve.

None of the signs described above are absolute, and the experienced clinician uses an amalgam of all the features to reach an opinion. Scoring systems such as the "risk of malignancy" index and neural networks have been used to try to weight the various factors and to replicate the decision-making process but so far have not outperformed the experienced clinician. They are an aide to those centers that do not image ovarian cancer often to decide on

subsequent management. Most scoring systems also include menopausal status and CA-125 level.

Outcome of Initial Ultrasound Assessment of an Adnexal Mass

- Benign lesion, no follow-up is needed.
- Benign, but needs removal or other treatment for symptoms.
- Most likely benign but follow-up ultrasound is needed after an interval to assess any change. It is important to use these repeat scans appropriately. Repeating scans every few months without making a decision is wasteful and unhelpful. Once an adnexal cyst is shown to be benign and there is no intention to intervene, there is no need for further scans.
- Indeterminate lesion, proceed to MR scan for further characterization.
- Malignant lesion, proceed to CT scan for confirmation and staging. This scan will also allow planning of image-guided biopsy if surgery is not thought appropriate.

Learning Points: Ultrasound

- Ultrasound is often the first imaging test in a suspected pelvic mass.
- Most masses can be correctly categorized as benign by ultrasound.
- The choice of follow-up ultrasound scan or proceeding to MR or CT depends on the initial ultrasound findings.

MR Imaging Technique for the Sonographically Indeterminate Adnexal Mass

US is the first line imaging investigation for the suspected adnexal mass and does an excellent job in characterizing the majority of women as either having no adnexal mass, a benign mass, or a malignant mass. But there is a significant minority of masses which are "indeterminate." An US indeterminate adnexal mass is defined as one which has complexity but which, after thorough interrogation including Doppler assessment, cannot be confidently placed into either the benign or malignant category or one for which the site of origin, from the ovary, uterus, or another pelvic structure, remains to be established.

Most referrals for MR imaging of a sonographically indeterminate mass are for:

- A solid (Fig. 5.3) or cystic-solid mass
- A cystic mass which contains either a thickened wall or an irregular mural or septal lesion, all morphologies which may represent malignancy

The majority of sonographically indeterminate adnexal masses are benign common entities such as hemorrhagic and inflammatory disease or common entities like uterine fibroids or ovarian teratomas which do not display their characteristic US features [2]. MR imaging has the greatest accuracy of any imaging test in assessment of the adnexal mass and complements US in this regard [3].

MR Technique [4]

The MR imaging technique used comprises two parts: a series of basic T1- and T2-weighted sequences to ascertain the site and signal characteristics of the mass and then selected problem-solving sequences tailored to the suspected nature of the mass to refine the diagnosis.

Patient preparation is minimal. There is no need for fasting. There is no need for a filled bladder; indeed if the patient expresses a desire to void before the examination, this is encouraged as an overfull bladder may result in a restless patient and images that are degraded by motion artifact. Use of a smooth muscle relaxant such as intravenous Buscopan (hyoscine butylbromide) or glucagon is advised.

The basic series of MR imaging should comprise a T2-weighted (T2W) sagittal sequence of the pelvis to assess the uterus and the position of the adnexal mass and a pair of T1-weighted (T1W) and T2W sequences covering the indeterminate adnexal mass in an orthogonal plane with similar slice thickness to allow precise comparison of its tissue characteristics,

to further detail its anatomic location, and to suggest its organ of origin.

The choice of which orthogonal plane is used for this pair of sequences is left to the discretion and preference of the supervising radiographer/technologist or radiologist. If the mass is noted to lie lateral to the uterus on the initial sagittal T2W sequence, its relationship to the uterus may be better shown on coronal or coronal oblique oriented imaging and if it lies above or behind the uterus on axial or axial oblique oriented imaging. Our own preference for this pair of sequences uses the plane of the long axis of the uterus, parallel to the endometrial stripe. We term this the "ovarian axis."

In order to determine the site of origin of the indeterminate adnexal mass, it is important to identify both ovaries, presuming there has not been prior resection. Definition of two normal ovaries separate from the mass indicates that the mass is either uterine or arising within one of its suspensory ligaments, tubal, or even non-gynecological.

MR Imaging Features [4]

Following the basic T1W and T2W sequences described above, the likely site and origin of the mass and its signal characteristics are established. Problem-solving sequences are then chosen to refine the diagnosis based upon key signal characteristics and morphology. Masses are divided on this basis into three broad categories for which a specific problem-solving sequence is applied:

- *T1 bright masses* containing T1 high signal require additional fat-suppressed T1-weighted (FST1W) imaging using chemical presaturation. Fat within a mature cystic teratoma will show signal loss becoming "dark" on the FST1W images. Other masses with T1 "bright" signal include blood products within hemorrhagic masses including endometriomas or masses which have undergone internal bleeding or torsion; mucin and other proteinaceous material within some cystic neoplasms; malignant neoplasms with hemorrhagic change; and, rarely, melanin within melanoma metastases. All these remain "bright" on the FST1W images.
- *T2 solid masses* require further assessment to determine if they arise from the ovary or uterus. Their signal is either similar to or lower than skeletal muscle, T2 "dark" solid masses, or equal to/higher than muscle, T2 "intermediate" solid masses. In some cases, application of the oblique "ovarian axis" provides a diagnosis. Others may require additional T2-weighted oblique imaging to identify the relationship with the uterus, i.e., distinction of uterine leiomyoma from ovarian fibroma. Many T2 "dark" masses can be confidently diagnosed as a leiomyoma or fibroma with simple T2W sequences (Fig. 5.3). Some solid ovarian masses with inhomogeneous low T2 signal or intermediate T2 signal require further assessment with contrast (gadolinium) enhancement T1-weighted images (CET1W). Fibromas show no or minimal enhancement. Masses with significant enhancement should be regarded as potentially malignant. An alternative strategy is to use diffusion-weighted imaging (DWI).
- *Complex cystic or cystic-solid masses* within the spectrum of cystadenoma through borderline to malignant neoplasm require CET1W imaging to determine the presence of neoplastic tissue. This applies not only to classical cystic-solid masses but also to other masses with "worrying" solid components. Some mature teratomas and complex endometriomas contain complex mural or internal solid elements. These should raise concern as both entities may rarely undergo malignant change. Clot retraction and fibrotic nodules in hemorrhagic and postinfective/inflammatory masses do not show enhancement.

It is rare for all three problem-solving sequences to be required, but when there is no radiologist to supervise the study, it may be necessary to prescribe these to ensure a diagnosis. In many cases, a single uncertainty exists after US assessment: the mass is solid but its origin from the ovary or uterus is unclear; there is a nodule, but whether this is a blood clot or solid tumor is unclear. Many US indeterminate masses, which are most commonly hemorrhagic/endometriotic cysts, atypical dermoid tumors with minimal fat or solid masses in the leiomyoma/fibroma

spectrum, can be characterized without recourse to the additional cost of CET1W imaging.

Novel MR Techniques [5]

Two research applications of MR imaging which show promise are dynamic contrast-enhanced MR imaging (DCEMR) and DWI. DCEMR imaging obtains numerous acquisitions during the first couple of minutes after contrast injection to define an enhancement profile of the mass or a part of it. This can be performed in a variety of ways, some of which are not widely available. DCEMR can be included in the interval between the T1W and CET1W sequences. Recent data suggest that enhancement profiles within solid vegetations can help in identifying lesions which require cancer surgery by distinction of benign from borderline and malignant disease.

DWI looks at the behavior or water molecules in the local environment of the mass and the restriction of this movement by cellular density. Masses which have high cellular density have high signal and those which are predominantly fibrous do not. Thus using DWI, masses with low signal on images with b values of 750–1,000 are almost always benign.

Follow-up Using US

An alternative to MR imaging for indeterminate masses which raise lesser concern is interval reexamination with US. This ploy is widely used in clinical practice and is particularly appropriate for the assessment of premenopausal women when it is considered likely that the abnormality represents a physiological variant such as hemorrhage into a corpus luteum or a functional cyst or a complication of common conditions such as endometriosis or pelvic inflammatory disease.

It is usually recommended that the repeat US examination takes place after two menstrual periods and ideally within the first 10 days of the menstrual cycle. Thus, follow-up is typically within 6–8 weeks.

For a postmenopausal woman, this ploy is less valuable as adnexal hemorrhage and inflammation are much less likely. Conversely, it is uncommon for postmenopausal masses to change their character, so once this has been achieved, there is no case for serial follow-up.

The Adnexal Incidentaloma

Indeterminate adnexal masses arise not just from US examinations but from CT. Such findings are termed "incidentalomas." These are increasingly found during the investigation of gastrointestinal and urinary tract symptoms. Computed tomography (CT) has become the mainstay of imaging of the abdomen, and specialized techniques like CTC and CT urography (CTU), which show a whole range of internal organs as well as the system of interest, have supplanted traditional contrast radiography which showed only parts of the organ system of interest.

The result is an explosion of incidental findings including adnexal masses. These masses being found on examinations performed for other suspected cancer symptoms are often referred on to the gynecological cancer MDTM in a situation of "high anxiety." The vast majority will be benign. Our usual practice is to refer CT incidentalomas for US unless it is the judgment of the reporting or reviewing radiologist that US is unlikely to resolve the problem.

A small minority of adnexal masses remain indeterminate even after interrogation with MR imaging. For others, there is a worrying elevation of the CA125 level to several times the upper limit of normal. Here, our policy is for further discussion in our weekly MDTM. A mass which cannot be confidently diagnosed as benign after US and MR imaging should undergo operation by a gynecological oncologist.

Learning Points: MR Imaging at Diagnosis

- MR imaging is the most accurate presurgical test for the evaluation of the adnexal mass.
- The majority of complex adnexal masses and adnexal incidentalomas are benign.

- Key problem-solving MR sequences are fat-suppressed T1W and gadolinium-enhanced T1W.
- Masses which show gadolinium enhancement should be regarded as malignant.
- Exceptions include some benign stromal masses, chronic infection, and acute inflammatory disease, the latter of which can be distinguished clinically.
- Diffusion-weighted imaging (DWI) is valuable as solid masses with low signal of $b = 750–1{,}000$ images are usually benign.

CT at Initial Diagnosis and in Staging Prior to Surgery or Neoadjuvant Chemotherapy

CT is used when, after assessment with US, the working diagnosis is ovarian cancer and staging assessment is required (Fig. 5.4). Thus, women referred for CT have either:

- A malignant looking ovarian mass
- A malignant ovarian mass and evidence of peritoneal spread (the presence of ascites almost always indicates this) or pleural spread
- Evidence of peritoneal carcinomatosis without a definite primary tumor site

CT is used to confirm the US impression of malignancy and to assess the sites and extent of any metastatic disease (Table 5.1). However, some other cancers may mimic ovarian cancer by producing peritoneal and ovarian metastases. Thus, the purpose of CT examination is:

- To confirm the US impression of ovarian malignancy
- To evaluate sites of metastasis and consider options for cytoreductive surgery
- To look for alternative diagnoses which could mimic ovarian cancer
- To consider the necessity and options for image-guided core biopsy or other diagnostic methods

For women believed to have peritoneal spread of ovarian cancer (and two thirds of women with ovarian cancer present this way), the key question after CT is whether radical cytoreductive surgery is possible and appropriate (Table 5.1, Fig. 5.2) [6]. This is a judgment to be made in the setting of the MDTM. Surgeons differ in their approach to cytoreductive surgery, and the information presented from the CT staging assessment needs to be tailored to this.

Table 5.1 Problematic sites of metastasis when considering cytoreductive surgery[a]

Pelvis
Retroperitoneal presacral disease
Pelvis sidewall invasion
The sigmoid mesentery[b]
Abdomen
Lymph node enlargement above the renal hilum
Abdominal wall invasion
The small bowel mesentery
Parenchymal liver metastasis
Deep subcapsular liver metastasis
Disease at the porta hepatis, intersegmental fissure, and gall bladder fossa
Diaphragmatic disease
Supracolic disease in the lesser sac, gastrosplenic and gastrohepatic ligaments
Chest and neck
Any deposit >2 cm

[a]Any deposit left greater than 1 cm is viewed as a failure of cytoreduction
[b]Involvement of this may require a colostomy to effect cytoreduction

Other key considerations are fitness for this major abdominal surgery (patient performance status) and a past medical history of a cancer whose metastatic spread can mimic ovarian cancer, e.g., the GI tract or breast. When there is doubt about the diagnosis, IGCB should be considered. When surgery is not deemed appropriate again, IGCB should follow to provide a site-specific diagnosis to guide oncologic therapy.

CT Technique

The standard CT technique for examining women with suspected ovarian cancer includes the abdomen and pelvis. Our preference is for the use of positive oral contrast. The patient fasts for 4 hours prior to the CT examination. On arrival in the CT department, the patient is asked to drink slowly and steadily 1,000 ml of 3 % Gastrografin over the hour prior to examination with the final 200 ml of this taken immediately prior to the

examination in order to distend and opacify the stomach and duodenum.

Intravenous contrast is routinely administered, typically 100 ml of a 300 mg iodine strength nonionic agent at 3 ml/s using a pump injector. The image acquisition is timed to coincide with maximal portal venous enhancement beginning at 65 s and covers from the domes of the diaphragm to the lower border of the pubic symphysis. With the advent of multidetector CT, it is possible to reconstruct high-quality reformatted images in the coronal and sagittal plane, and imaging parameters should be chosen to allow this.

The chest is only examined in patients at initial staging when pleural effusion, lung metastasis, or mediastinal lymphadenopathy have been discovered by chest radiography. It is not usual to request a chest radiograph in the absence of chest symptoms. When a pleural effusion is evident on the abdominal CT at the lung bases, the chest is also examined.

It is important to be aware of patients with renal impairment and to take measures to minimize contrast medium nephrotoxicity (CMN). These patients at risk should receive a small dose of either nonionic iso-osmolar dimeric or nonionic low osmolar monomeric contrast medium and intravenous fluid. Intravenous infusion (1 ml/kg body weight/h) of 0.9 % saline starting 4 hours before contrast injection and continuing for at least 12 hours afterwards is effective in reducing the incidence of CMN.

CT Features of Untreated Ovarian Cancer

The CT features of epithelial ovarian cancer are those of the primary tumor(s) and those of the metastatic spread. A variety of "typical" primary tumor patterns are seen which are similar to the morphologies shown by US: (a) a cystic mass with a solid mural nodule, (b) circumferential mural thickening and irregularity, (c) multilocularity with differing contents, and (d) multiple irregular internal solid elements. However, occasionally ovarian cancer may result in a predominantly solid mass with areas of necrosis. Calcifications and contrast enhancement may be present in the cyst wall or within solid tumor components.

The CT appearance of ovarian metastases may be indistinguishable from that of primary ovarian cancer. Both may produce bilateral masses. In the RDOG study, the only factor favoring primary ovarian cancer was multilocularity as shown by US or MR imaging. This was not a significant feature for CT. The stomach, colon, appendix, and pancreas are within the examination volume and should be inspected as potential primary cancer sites within the abdomen.

Primary ovarian cancers are most frequently found in the adnexal region or pouch of Douglas (rectouterine recess) displacing and compressing the uterus, bladder, and rectum/sigmoid colon. If the ovarian mass continues to enlarge into the abdomen, it lies above the bladder displacing pelvic small bowel. Eventually, the mass may reach the undersurface of the liver.

Metastatic spread is predominantly via the peritoneal cavity usually with ascites but also via lymphatics to the pelvic and para-aortic groups and by hematogenous spread to the liver. Patterns of spread identified within the pelvis (stage II) by CT are involvement of small and large bowel, the pelvic sidewall with encasement of the iliac veins leading to thrombosis, and of the pelvic ureter with resultant hydronephrosis. Hydronephrosis is, however, more commonly due to simple mass effect upon the pelvic ureter. Metastatic spread to the abdomen (stage III) may be manifest as peritoneal and mesenteric masses and omental cakes and there may be involvement of the abdominal wall, notably at the umbilicus, the 'Sister Mary Joseph' nodule. Involvement of the surface of the liver and spleen is classified as stage III disease but parenchymal deposits within the liver upstage the patient to stage IV.

Detection of peritoneal seedlings is easier in the presence of ascites. However, CT can detect the calcified tumor implants containing psammoma bodies from serous cystadenocarcinoma of the ovary even in the absence of ascites. Conversely, densely calcified tumor implants from serous tumors may be mistaken for bowel containing oral contrast. CT can detect 50 % of

implants that are 5 mm or more in size. Detection of these and smaller peritoneal seedlings remains the province of surgery.

Current Best Evidence Regarding Presurgical Imaging

In the late 1990s, the Radiology Diagnostic Oncology Group (RDOG) conducted a major multicenter diagnostic imaging study of women prior to ovarian cancer surgery and published its findings in three landmark papers [7–9]. This work has not been repeated in the modern imaging era. The RDOG studies compared US, CT, and MR imaging in 280 women, evaluating these modalities for cancer diagnosis and staging. In the study, 189 women had unilateral masses and 91 had bilateral masses. Only 114 of the 280 women had ovarian cancer, and of these, 27 were not primary ovarian cancer but other malignancies metastatic to the ovary, some of which demand entirely different cancer management. Women with a history of malignancy were excluded so the risk of confusion between primary ovarian cancer and other cancers metastatic to the ovaries was less than in routine clinical practice.

A number of lessons have been learned and reemphasized by these important studies. They have shown that both CT and MR imaging are superior to US in assessment of the nature of ovarian masses, with the highest accuracy for MR imaging. In assessment of the stage of disease, all had similar accuracy (0.91) since the presence of ascites effectively predicted peritoneal spread of tumor. However, in determination of the sites and extent of this metastatic tumor, US was inferior to both CT and MR imaging. A particular problem for US was in depiction of peritoneal metastases. The ready availability of CT makes it the investigation of choice for planning surgery in women believed to have metastatic spread of ovarian cancer. CT can replace urography and barium studies for assessment of hollow organ involvement and in most cases is the only imaging study required to plan management.

CT remains inferior to surgical staging in detection of tiny peritoneal, omental, and mesenteric nodules even with meticulous technique using multidetector CT technology not available at the time of the RDOG studies. But this is not its role. CT defines and alters patient care at the other end of the spectrum of ovarian cancer (Fig. 5.2). In the presence of bulky metastatic tumor, CT predicts when cytoreductive surgery is likely to be incomplete by defining sites of unresectable tumor. CT indicates when the gynecologist may require assistance from other surgical colleagues to achieve effective debulking, when, for example, there is involvement of ureters, pelvic small bowel, or colon. The need for colostomy may be highlighted. Bulky disease in the supracolic compartment around the spleen and stomach, within suprarenal lymph nodes, and affecting the subdiaphragmatic recesses and parenchyma of the liver is usually beyond the scope of surgery. CT may highlight invasion of the abdominal wall which may compromise or contraindicate attempts at resection.

CT provides the surgeon with the detail required to discuss surgical and other therapeutic options with the patient and her carers. A variety of CT schemes have been devised to judge the tumor extent at key sites. Almost any amount of tumor can be resected, but at what cost? And with what benefit? Rather the surgeon needs to be alerted to the actual extent of disease at key sites and the likelihood of success. CT can do this and the information presented by the radiologist needs to focus upon and address these points.

For some years women with very advanced disease at presentation or poor performance status have been treated with primary (neoadjuvant) chemotherapy in the hope that subsequent surgery may be possible. This approach has been formally investigated in the EORTC 55971 study and the MRC UK CHORUS (Chemotherapy OR Upfront Surgery) study. These address the timing of surgery relative to chemotherapy for newly diagnosed ovarian cancer. Women felt to be "operable" at diagnosis were randomized to receive either surgery followed by chemotherapy or neoadjuvant therapy followed by IDS. The results from the EORTC 55971 study

are clear: there were similar outcomes for the two randomized arms; there was lower surgical morbidity in the IDS arm and more complete debulking was achieved [10]. The CHORUS study has confirmed the similar outcome of women in the two arms of treatment but shown an even greater rate of surgical complications in the women having primary surgery.

The management implications of these studies are significant: at first sight, the pivotal role of surgery in initial management is challenged. There has been much debate and some criticism of the study findings and recommendations, but it seems clear that "use of neoadjuvant chemotherapy is a safe alternative in this group of patients and does not compromise the standard of care."

There are also significant implications for imaging and investigation of these women with suspected ovarian cancer. Embarking upon neoadjuvant chemotherapy demands a confident histological diagnosis and this can be provided by image-guided core biopsy (IGCB) [11]. This becomes more important as the investigation of chemotherapy regimens specific to the different subtypes of ovarian cancer are being established. Put simply a diagnosis of adenocarcinoma based on cytological evaluation of ascitic fluid is increasingly insufficient. Other cytological techniques such as preparation of a cell block specimen are untested in this regard.

Diagnostic methods for assessment of the cause of peritoneal carcinomatosis include:

- Tumor markers with a ratio of CA125 to CEA of >20 considered suggestive of ovarian cancer
- Imaging assessment
- Cytological assessment of peritoneal fluid
- Image-guided core biopsy (IGCB)
- Laparoscopic biopsy

IGCB is an effective, safe, and well-tolerated alternative to surgery (minilaparotomy, laparoscopy) in providing a definitive histological diagnosis when cancer surgery is not considered appropriate. IGCB using CT or US guidance is a valuable and useful alternative to laparoscopy or exploratory surgery in the following circumstances:

- In women believed to have ovarian cancer but with poor performance status or with advanced disease believed beyond the scope of primary cytoreductive surgery
- In women with a history of cancer whose metastases may mimic ovarian cancer (e.g., breast, GI tract, melanoma)
- When there is diagnostic uncertainty, e.g., unusual imaging patterns or with an unusual tumor marker profile

In a woman with undiagnosed peritoneal carcinomatosis, IGCB should precede an exhaustive (and potentially hazardous and unpleasant) series of investigation of potential primary sites such as upper and lower bowel endoscopy. Its findings can focus the search for the primary tumor when appropriate. Management of such women is best discussed in a multidisciplinary setting.

One cause of diagnostic uncertainty recognized in recent years is primary peritoneal carcinoma (PPC), which arises from the peritoneal surfaces as a papillary serous tumor of Mullerian duct origin. This entity is managed in an identical manner to primary ovarian cancer with cytoreductive surgery and platinum-based chemotherapy, yet has some differences in imaging findings. The typical CT features of PPC are of ascites, omental, and peritoneal masses, which may be calcified but with normal-sized ovaries (Fig. 5.5) [12]. PPC was initially recognized as a subset of women with peritoneal carcinomatosis whose prognosis was more favorable. Median survival was 23 months with PPC compared with 3–4 months for peritoneal carcinomatosis related to non-gynecological malignancy. The diagnosis of PPC was made at laparotomy in those series, but it is a diagnosis which can be suggested on the basis of imaging and confirmed using image-guided needle core biopsy. Both ovarian cancer and PPC may occur in women with the BRCA1 and BRCA2 gene mutations for breast cancer. The treatment options and prognosis differ markedly between ovarian cancer/PPC and abdominal recurrence of breast cancer. Thus, in a woman with prior breast cancer with peritoneal carcinomatosis, IGCB allows this distinction to be made with confidence allowing comparison with tissue from the breast biopsy or specimen from the initial diagnosis.

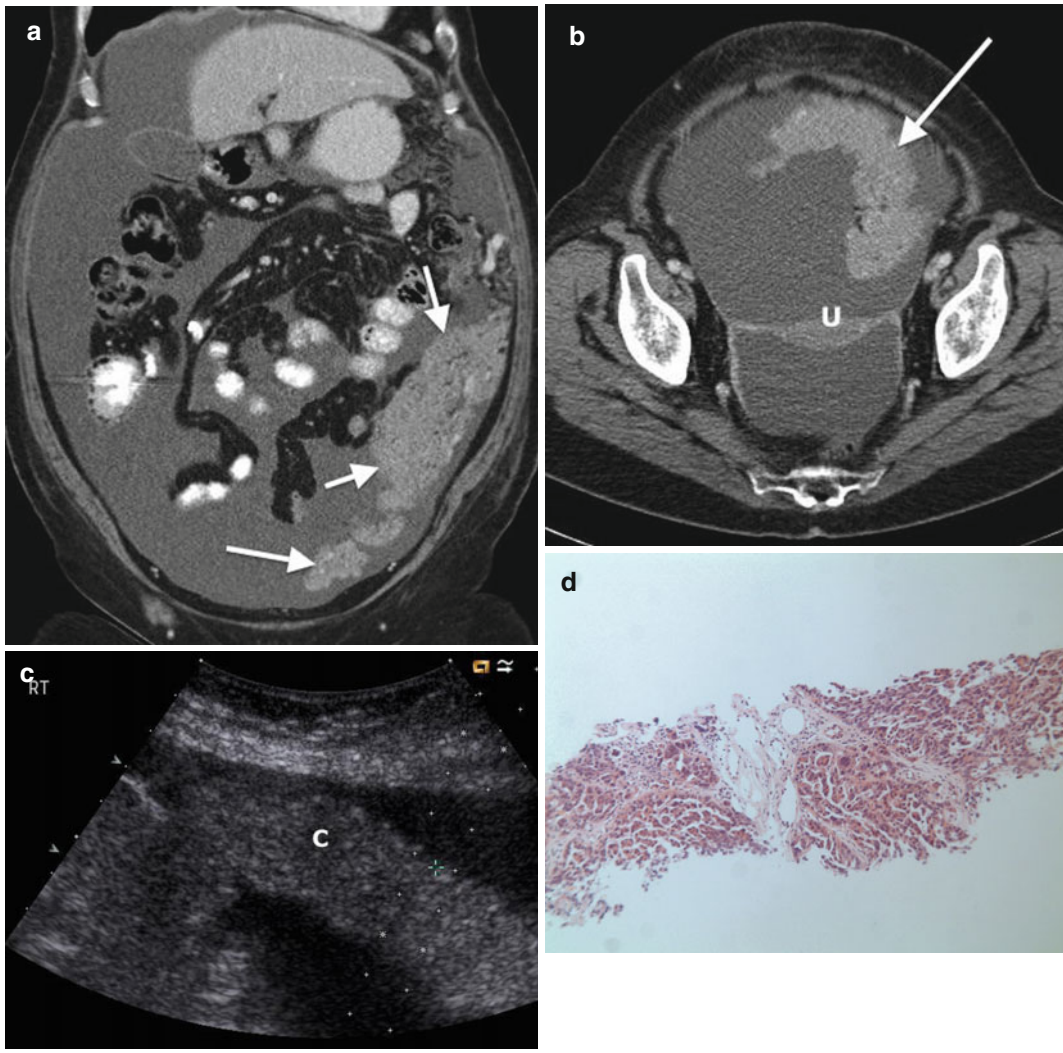

Fig. 5.5 (**a**) coronal reformatted contrast enhanced CT showing gross ascites and a large omental cake (*arrows*) and (**b**) axial contrast enhanced CT of the pelvis showing the cake (*arrow*) a small post-menopausal uterus (*U*) but no adnexal masses. This woman had no apparent primary tumour within the abdomen or pelvis but a history of breast cancer. After MDTM discussion (**c**) US guided core biopsy was performed from the cake (*C*) and (**d**) the findings were of a high-grade serous carcinoma and a diagnosis of primary peritoneal carcinoma

Technique for CT-Guided Core Biopsy of the Peritoneum [11]

Criteria for CT-guided biopsy are: (1) the presence of omental, peritoneal, or pelvic mass allowing core biopsy on diagnostic imaging; (2) no bleeding diathesis with platelet count $>10\times10^9$/L and INR (International Normalized Ratio) <1.4; and (3) a decision made after MDTM review that obtaining a definitive diagnosis by nonsurgical means was required to plan further treatment and cannot be provided using US guidance.

The image-guided biopsy is performed as a separate procedure after MDTM review of the diagnostic studies. For the biopsy, CT is performed with only oral contrast preparation and after a limited number of localizing sections planned from the prior study. When the peritoneal disease is heavily calcified on the

diagnostic study, water is preferable to opacify the small bowel. An 18G cutting needle using a spring-loaded device is used (Temno, Bauer Medical International SA, Dominican Republic). The number of needle passes made is judged by the supervising radiologist to provide the equivalent of two full needle cores of solid material for the pathologist. From a solid omental cake, only two or three needle passes are required but with more whispy or nodular infiltrates up to six passes may be required. With such infiltrates, the specimen may be predominantly fatty and float on the formalin in the specimen bottle.

After an initial limited CT examination to localize the target mass, the biopsy procedure typically lasts 15–20 min. Aftercare includes bed rest for 6 hours with the measurement of blood pressure and pulse half hourly for 2 hours and then hourly for 4 hours after which the patient may eat and drink and become ambulant. The biopsy can be performed as a day-case procedure. For in-patients, the procedure may be combined with placement of an ascitic drain.

The vast majority of IGCBs can be performed with US guidance with the target planned from the diagnostic CT study (Fig. 5.5). We reserve CT guidance for small masses, those that are heavily calcified and those that are closely related to bowel.

Learning Points: CT Assessment

- CT is the most widely used test for assessment of tumor spread.
- It may identify significant complications including hydronephrosis, bowel obstruction, and venous thromboembolism.
- CT may identify other primary tumors in the GI tract whose metastatic spread masquerades as ovarian cancer.
- CT defines options for IGCB.

Ultrasound-Guided Core Biopsy of the Peritoneum and Adnexa

Ultrasound has advantages over CT in the acquisition of needle core biopsies (Fig. 5.5):

- Real-time visualization of the passage of the needle to the target.
- Alternate routes of access, such as transvaginal ultrasound-guided biopsies.
- Ultrasound is a cheaper modality than CT and the time required in the scan room is usually less than with CT.

The decision on the need for image-guided biopsy is made at multidisciplinary review. Seeding of tumor along a biopsy needle track or into the peritoneal cavity is a potential risk, though not one that has been quantified. Most reports are anecdotal. However, this perceived risk alters practice and routes of access. Percutaneous biopsies are not done in suspected malignancy unless there is already evidence of spread from the primary tumor. CT or MRI should have been done to stage the tumor prior to any biopsy. These scans are also vital for planning the biopsy procedure.

Omental Biopsy

I do this as an outpatient procedure with the patient spending only 30–40 min in the department after the biopsy. They should come with a companion who will take them home and be with them for the rest of the day. There is no preparation for the patient. Clotting needs to be corrected if they have been formally anticoagulated. Clopidogrel use is best discontinued for 10 days prior to the procedure, though not all authorities agree on this. Aspirin use is not a contraindication.

The prior imaging is scrutinized to familiarize the operator with the position and size of the omental cake. The operator then does a preliminary ultrasound scan to identify this omental cake and plan the point of skin puncture and needle path. The presence of ascites is useful as it helps outline the omental cake. Large omental deposits adopt a sheetlike appearance on both transverse and longitudinal ultrasound scan planes; this is in contrast to bowel that might look sheetlike in one plane but tubular in the other. Peristalsis and a multilayered wall echo also helps define bowel. Color Doppler is used to assess blood flow, which will be disordered in omental cake but ordered within bowel wall. Time spent in planning and preparation is never wasted and usually ensures the biopsy goes quickly and smoothly once started.

Skin cleansing, a sterile drape, and local anesthesia are used. A sterile ultrasound probe cover and a needle guide device are advised. A small nick in the skin with a scalpel blade and blunt dissection with small forceps facilitates passage of the biopsy needle. An 18 gauge, automated, spring-loaded biopsy needle is used. This is advanced to the target under direct real-time ultrasound visualization. The position of the needle tip and the firing of the cutting mechanism vary between manufacturers. Some require the tip to be placed adjacent to the target and the needle when fired advances 2 cm to take the sample, others need the needle to be positioned within the target tissue with the "tray" open, and when fired, the tray is closed but the needle does not advance. The operator needs to be familiar with the needle they are using.

The tissue core obtained should be inspected to see if it contains the firm white tissue that most malignant deposits exhibit. The number of needle passes required depends on the quality of the specimen obtained. The equivalent of two full length cores is usually enough to allow histology and the various immunohistochemistry stains.

Percutaneous omental biopsy is a very well-tolerated and safe procedure. No significant complications have been recorded in several series. Bowel is remarkably tolerant to the passage of an 18 gauge needle and no descriptions of bowel damage are reported; however, we have had one woman who required a minilaparotomy for perforated bowel. The occasional hematoma is also seen (1 out of 90 patients in one series).

Transvaginal Biopsy

Masses in the pelvis may be inaccessible to percutaneous biopsy because of intervening bowel, bladder, or vascular structures (Fig. 5.6). The transvaginal route of access can allow the tip of the probe to be placed directly adjacent to the target lesion so that only a few millimeters of tissue will have to be traversed by a needle to gain a sample.

The need for the biopsy and the pre-procedure planning and preparation are similar to that described above for omental biopsy. Transvaginal biopsy can be done as an outpatient procedure. The bladder needs to be empty. The patient lies in the usual position for a transvaginal scan. Stirrups are not needed. Some authorities advocate vaginal cleansing with povidone–iodine and a course of antibiotics as the vaginal route of access is not sterile. However, in my own practice, I have found that use of an antiseptic cream as the probe lubricant is sufficient and has not resulted in any infective complications in over one hundred procedures.

Careful discussion with the woman about the procedure and an appropriate reassuring environment is essential. This usually allows the biopsy to take place without undue discomfort. Some authorities prefer the use of conscious sedation, but if this approach is used, the woman will have to fast for 6 hours and remain in the department until fully recovered afterwards. My experience is that sedation is not necessary.

A transvaginal probe with a needle guide attachment is needed. The probe is introduced into the vagina and positioned with real-time ultrasound visualization so that the target is only a few millimeters from the probe – usually only the vaginal wall and the wall of the target lesion have to be traversed by the needle. Due consideration needs to be given to avoiding any intervening structures or blood vessels. A 21-gauge spinal needle is used to infiltrate local anesthetic into the vaginal wall. The probe is pushed gently but firmly in order to make the vaginal wall taut so that passage of a needle is easier. An 18-gauge automated spring-loaded biopsy needle is used to take the sample. One or two passes of the biopsy needle is usually sufficient.

Once the biopsy has been done, the woman can expect to see a little vaginal bleeding not unlike menstrual loss. Most are fit to leave after half an hour (unless they have been sedated). Complication rates are very low. Most of those in the literature are related to transvaginal drainage of endometriomas.

Learning Points

- Ultrasound-guided biopsy allows the passage of the needle to be seen in real time.
- Time spent prior to the biopsy in planning the route of access is never wasted. Prior

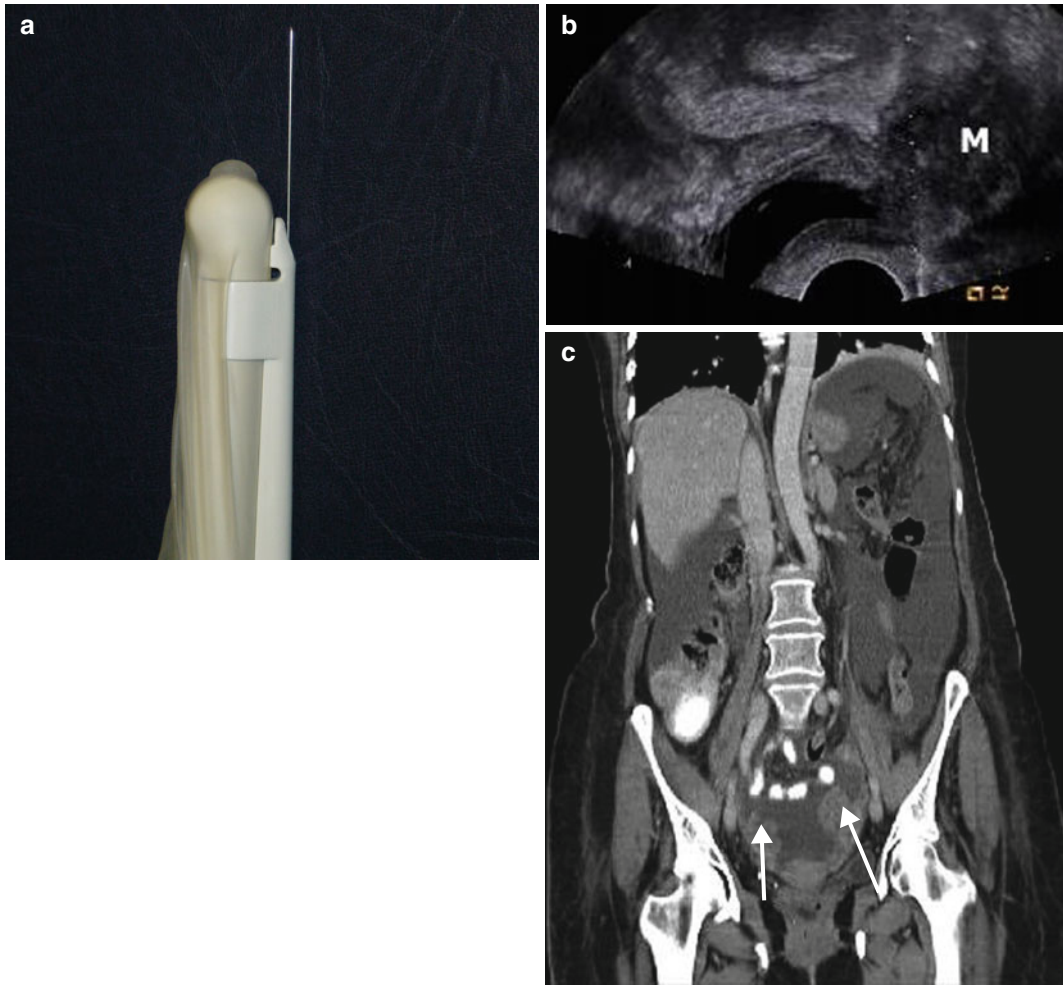

Fig. 5.6 (**a**) The probe used for transvaginal US guided core biopsy and (**b**) TVUS image showing the biopsy path into a solid adnexal mass (*M*) in a woman who presented with ascites, small volume peritoneal carcinomatosis and bilateral solid adnexal masses which were inaccessible from a percutaneous approach. Histology was of a high grade serous carcinoma. (**c**) Contrast enhanced CT showed large volume ascites but no percutaneous target mass only the deeply placed enlarged ovaries (*arrows*)

cross-sectional imaging needs to be scrutinized.

- Transvaginal biopsy is well tolerated and allows access to lesions that cannot be biopsied percutaneously.
- Transvaginal and percutaneous omental biopsy techniques have an excellent safety profile.

Pathology of Image-Guided Core Biopsies

Image-guided core biopsies (IGCB) of the omentum, peritoneum, and pelvic masses are becoming increasingly relied upon in the context of multidisciplinary team management of ovarian cancer. This may be approached by the

transabdominal or transvaginal route depending on the location of the tumor and the route considered most accessible and appropriate by the radiologist. There has, in the past few years, been a well-recognized shift in patients with advanced ovarian cancer being treated with three cycles of chemotherapy followed by interval debulking surgery rather than primary staging laparotomy. For this practice to be clinically appropriate, it is paramount to have the correct tissue diagnosis of the tumor prior to the commencement of treatment. A further complication is that if a diagnosis is not established at the outset, it may not be possible to make the diagnosis later once chemotherapy has started. This is because the effects of chemotherapeutic agents can significantly alter the morphology of the tumor so as to render them unclassifiable. In certain cases, the patient may be too ill to withstand primary surgery and require chemotherapy necessitating an image-guided core biopsy for further management. There have been extensive changes in the past few years of our understanding of ovarian cancer and the recognition that it is a heterogeneous disease with diverse morphological features, putative precursors, mutational profiles, prognosis, and response to therapy. As genetic profiling reveals specific characteristics of the different morphological subtypes, targeted therapy becomes more feasible and is likely to be the way forward in the management of ovarian cancer. The requirement for robust tissue diagnosis of a tumor subtype is therefore becoming increasingly important. The other major group where this technique is invaluable is in patients who already have a diagnosis of a malignancy which is usually non-gynecological, but they present with features suggestive of a disseminated "ovarian cancer." Here, the features are of an "omental cake" with a raised CA 125 and ascites. The radiological features are suggestive of a disseminated gynecological carcinoma. In these situations, it is important to exclude a metastasis from the previously diagnosed carcinoma.

Interpretation of the Core Biopsy

General Points

Prior to interpreting the biopsy, it is important to have obtained all the relevant clinical information regarding the patient's past medical history, the clinical impression, the tumor marker profile, and the radiological appearances of the disease process. The latter can be very useful in guiding the diagnostic pathway. All this information would be available during discussion at a gynecological oncology MDT. It is also advisable to retrieve any previous pathology relating to the patient which would enable comparison of the latest biopsy with any previous malignancy/malignancies.

The core biopsies are put into formalin and sent to the laboratory. The radiologist usually sends 2 or 3 cores. They are thin cores and therefore fix easily. They can be processed within 24–48 hours in the laboratory and this is usually the procedure in most laboratories. Hematoxylin and eosin-stained preparations can be assessed quickly, and the initial sections may be superficial and may not be representative of the tumor. If the tumor is not adequately represented on the initial sections, further levels can be requested, and at this time, it is prudent to request that material be saved from the intervening tissue for immunohistochemistry should it be required at a later stage. The material from a core biopsy is small but, if used judiciously, can provide a lot of valuable information with the added use of immunohistochemistry.

High-Grade Serous Carcinoma

Advanced ovarian cancer is usually dominated by type II ovarian cancers according to the dualistic model, and this subgroup predominantly comprises high-grade serous carcinoma which accounts for about 75 % of the ovarian cancers [13, 14]. It is by far the most common epithelial cancer likely to be encountered in biopsy material whether the disease distribution be

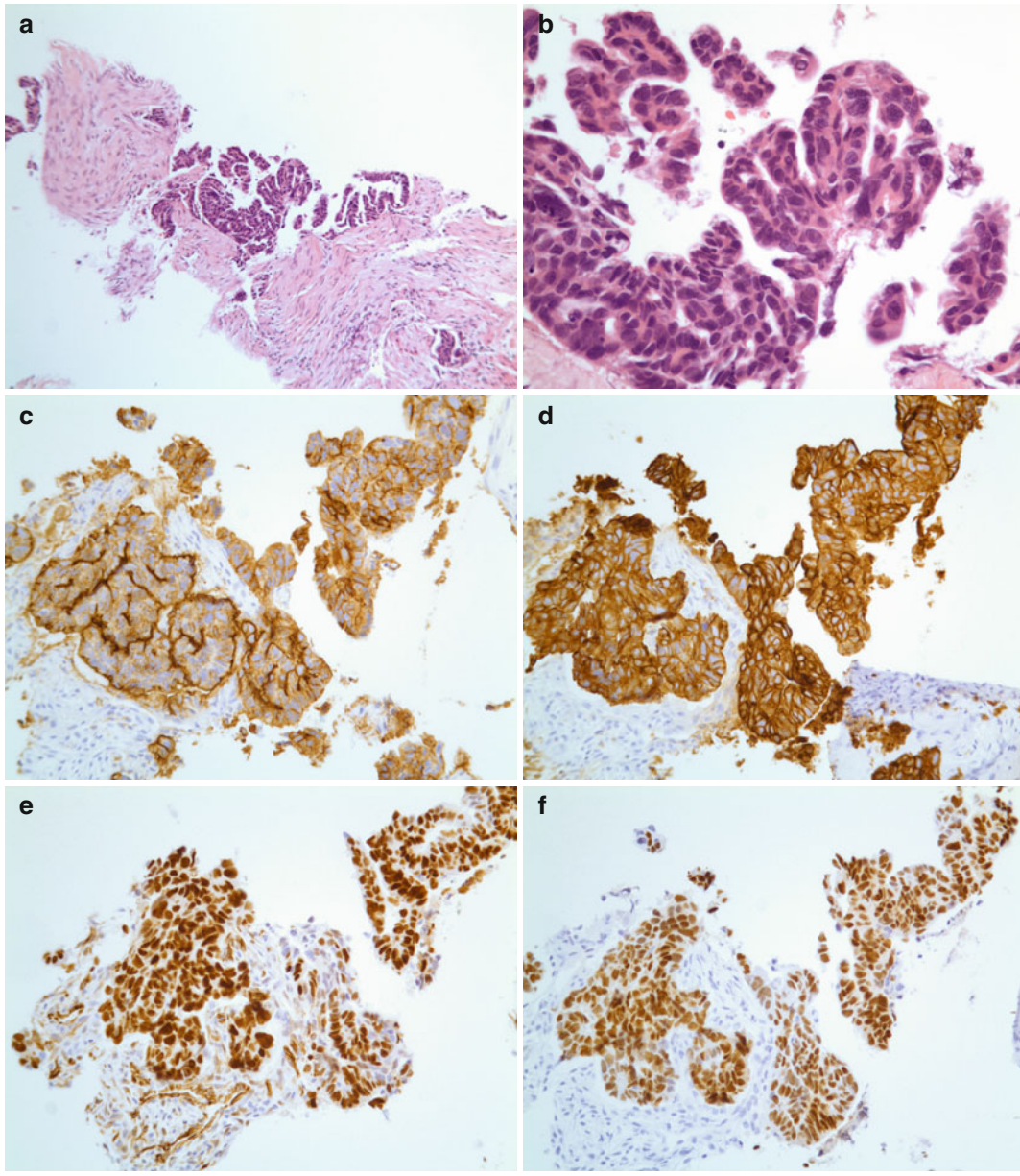

Fig. 5.7 (**a**) Low power view of high-grade serous carcinoma in a core biopsy of ovarian mass. (**b**) High power view of high-grade serous carcinoma in a core biopsy of ovarian mass. Note marked nuclear pleomorphism. (**c**) Uniform positive immunoreactivity for CA125 is seen within the tumour cells. (**d**) The tumour shows positive immunoreactivity for cytokeratin 7. (**e**) There is uniform positive immunoreactivity for WT-1. (**f**) The tumour shows strong positive immunoreactivity for oestrogen receptor

suggestive of an advanced ovarian cancer or a primary peritoneal carcinoma. The biopsy may represent only a solid component of serous carcinoma or a mixed solid and papillary pattern with high-grade cytological features. When a papillary component is identified, the diagnosis of the tumor can be made with ease given all the other clinicopathological parameters (Fig. 5.7a, b). When the biopsy represents only the solid component of a high-grade serous

carcinoma, the diagnosis can be difficult. Further immunohistochemistry is rarely needed when the typical histological features of a high-grade serous carcinoma are seen, but when the solid component is seen in isolation, immunohistochemistry for confirmation of the diagnosis becomes necessary. In such situations, immunohistochemistry using a panel of antibodies may be useful, cytokeratin 7, CA125, estrogen receptor (positive in about 60 % of cases), and WT-1 (nuclear positivity in the majority of cases) (Fig. 5.8c–f). A high-grade solid component may exhibit a predominant glandular morphology and resemble high-grade endometrioid adenocarcinoma. In this case, a metastatic colorectal carcinoma should be excluded with the addition of cytokeratin 20, CEA-M, and CDX2 to the immunohistochemical panel. Unfortunately, patients do not always present with a history of symptoms that could be related to primary gastrointestinal origin as on occasion the gastrointestinal primary may be undiagnosed when the patient presents with their "gynecological cancer" [15].

Low-Grade Serous Carcinoma

Low-grade serous carcinoma may on occasion be represented on biopsy material. Architecturally, the tumor characteristically comprises micropapillae and nests of cells that infiltrate the stroma in a haphazard pattern. The micropapillae are small and either lack fibrovascular cores or have very thin, delicate fibrovascular cores [16]. They are frequently surrounded by a clear space or cleft. Psammoma bodies are often seen. Necrosis is not a feature. The tumor shows nuclei that are uniform, small, and round to oval. The chromatin is even. Small nucleoli may be seen. The criteria for the distinction of high-grade serous carcinoma from low-grade serous carcinoma can be difficult to apply when the material is limited. Mitoses are usually scarce with a mean of four mitotic figures per 10 HPFs (range 1–12 MFs/10 HPF); this is not always helpful as 10 HPF of tumor are rarely encountered on biopsy material [17]. There is minimal variation in nuclear size, but should this become conspicuous and exceed >3:1 variation in nuclear size and shape, a diagnosis of high-grade serous carcinoma should be made. Multiple levels can sometimes reveal further tumor in the deeper sections and allow assessment of more material which helps make the correct diagnosis. Ki-67 proliferation index made be a useful adjunct in these situations where a low proliferation index would favor the diagnosis of a low-grade serous carcinoma. In one study proliferation indices for low-grade serous carcinoma was found to be 23 and 55 % for high-grade serous carcinoma [18]. While the theoretical possibility of distinguishing an "implant" from a low-grade serous carcinoma exists, in practice this has never been an issue. Implants are too small to be identified on CT and are, as such, not targeted for IGCB.

Mucinous Lesions

Omental biopsies on occasion may show extracellular mucous dissecting through a collagenous stroma. The clinical syndrome is described as a "pseudomyxoma peritonei." The mucous may be acellular or contain scattered atypical cells; when the latter is the case, then the possibility of an appendiceal neoplasm must be considered in conjunction with a benign or borderline appearing mucinous neoplasm of the ovary. In these situations, the case must be discussed at the MDT with all the available clinical and radiological information. It must be remembered that if the biopsy does not include cells within the mucous, this may represent a sampling problem rather than reflect the true pathology of the lesion.

In the vast majority of cases, the mucinous lesions biopsied are tumors that masquerade as ovarian carcinoma in their distribution with an involved "omental cake." These tumors are either poorly differentiated mucinous carcinomas of gastric origin or pancreatic adenocarcinoma metastatic to the ovary/omentum. The gastric carcinomas most commonly demonstrate signet ring morphology with the nuclei pushed to one side and large amounts of intracellular mucin distending the cell. These "signet" ring cells may be seen in isolation or as nests and clusters at times showing gland formation (Fig. 5.8a, b). Alternatively,

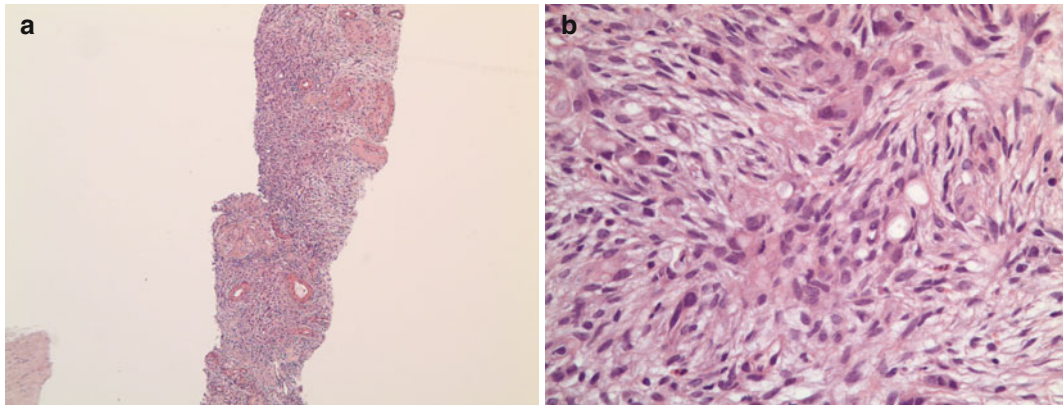

Fig. 5.8 (**a**) Core biopsy of ovarian mass showing a cellular neoplasm (low power). (**b**) The cellular neoplasm comprises numerous "signet" ring cells typical of metastatic carcinoma of gastrointestinal origin and most likely to be gastric in origin (high power)

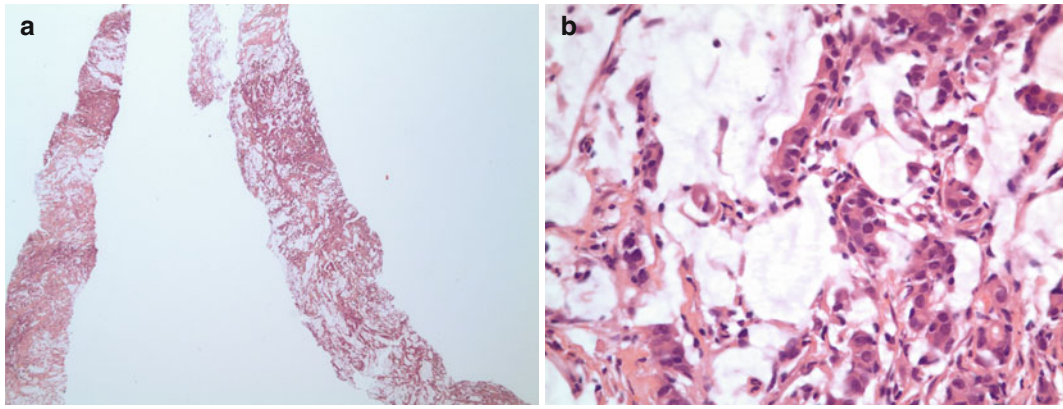

Fig. 5.9 (**a**) Core biopsy demonstrating groups of cells floating in copious amounts of extracellular mucin (low power). (**b**) Core biopsy demonstrating glandular acini associated with extracellular mucin in large amounts (high power)

these groups of cells may be seen floating in pools of extracellular mucin (Fig. 5.9) or associated with a desmoplastic stroma. The latter is very difficult to appreciate in small biopsy material. Here, it is the morphology which prompts the pathologist to think of non-ovarian primary origin. The primary tumor may not be located in the stomach but could originate in the large intestine particularly the caecum and rarely even the appendix.

Pancreatic adenocarcinomas show variably sized glands; some form tubules with a low epithelial lining, others may be cystic and angulated. This wide variation in shape, size, and structure of the glands is often a significant characteristic that maybe seen within a small biopsy. The epithelial lining of the glands can vary from showing minimal atypia to extremes of cytological atypia with some cells showing vacuolation of the cytoplasm reminiscent of secretory change. Occasionally single cells may also be seen lying within the stroma which can be variably desmoplastic. Immunohistochemistry shows positive immunoreactivity for cytokeratin 7, and sometimes focal positivity may be encountered with cytokeratin 20. Dpc4 expression is lost in 50 % of pancreatic adenocarcinomas, and this may be helpful diagnostically. In the presence of the above histological features within an omental biopsy, particularly if there is bilateral ovarian enlargement, a metastatic origin must be considered and excluded.

Metastatic cholangiocarcinoma forms small tubular glands, lined by low mucinous epithelium, and exhibits variable cytological atypia which can be quite marked at times. The stroma can be fibrous or fibromyxoid. Unfortunately, it may be difficult to appreciate the stromal changes in a small biopsy.

Primary mucinous carcinomas of the ovary are usually stage I at presentation, and if the history is of a first presentation with a disseminated mucinous adenocarcinoma mimicking a primary ovarian mucinous carcinoma, it is far more likely to be a metastatic mucinous carcinoma. This, however, may not be the case if it is a recurrence of a previous ovarian mucinous adenocarcinoma. Immunohistochemistry is of little value as there is overlap in the immunoreactivity of ovarian mucinous carcinoma and cholangiocarcinoma. Imaging is again very valuable in identifying any evidence of thickening or abnormalities of the biliary tract.

Colorectal Metastasis

In the last couple of decades, pathologists have become very aware of the morphological features of colorectal carcinoma metastasizing to the ovary and presenting as an ovarian carcinoma with an omental cake and ascites. As mentioned above, the metastasis may present prior to the presentation of the colorectal tumor with no preceding symptoms. On biopsy material, it can be very difficult if one does not consider the possibility of a metastasis. This is most problematic on transvaginal biopsies of ovarian masses where an "endometrioid" appearance to the tumor is seen and there is a lack of squamous metaplasia and clear cell change with extensive necrosis not unlike the "dirty" necrosis characteristic of colorectal metastases. On biopsy material, sometimes only parts of cystically dilated glands are included. The cytological atypia however of the epithelial lining is far greater than one would see in an endometrioid adenocarcinoma of low grade with well-formed glandular architecture. Immunohistochemistry for cytokeratin 7 and 20, CEA-M, and CA 125 with CDX2 usually resolves the problem.

Breast Metastases

Patients who have had a previous diagnosis of a primary breast carcinoma and then present with the picture of a primary ovarian or peritoneal carcinoma could either have a recurrence of their previous breast tumor or present with a new ovarian/primary peritoneal carcinoma. MDT discussion with familiarity of the radiological appearances together with the tumor marker profile is important. Bilateral ovarian masses of small size in the absence of a raised serum CA 125 are likely to represent recurrence of the previous breast carcinoma, and it is important to review the previous sections from the breast carcinoma and compare the new biopsy with the previous. Infiltrating lobular carcinoma is not usually problematic to diagnose on biopsy material but infiltrating duct carcinoma can on biopsy material pose problems where solid areas and a slight papillary morphology may resemble high-grade serous carcinoma. Unfortunately, immunohistochemistry shows overlap in several markers as cytokeratin 7, ER, PR, and CA125 can be positive in both. WT-1 however would be expressed in high-grade serous carcinoma as would PAX 8 [19], which would not be present in breast carcinoma. These latter two markers are helpful markers for confirmation of the primary site of origin of the tumor.

Cervical Metastasis

Metastatic adenocarcinoma of cervical origin can also mimic a primary ovarian adenocarcinoma, and this is particularly the case in biopsy material. It must be reemphasized here that it is imperative to know the past history of the patient with respect to previous cervical pathology even if a previous cervical lesion was reported as high-grade CGIN with no or very focal early invasion or foci suspicious of invasion. Ovarian metastases of cervical adenocarcinoma are probably under reported due to the lack of recognition by the pathologist and unawareness of the previous pathology. The tumors may have a "villo-glandular" morphology resembling an endometrioid adenocarcinoma (Fig. 5.10a, b) or a mucinous tumor of the ovary with a confluent, expansile pattern of invasion.

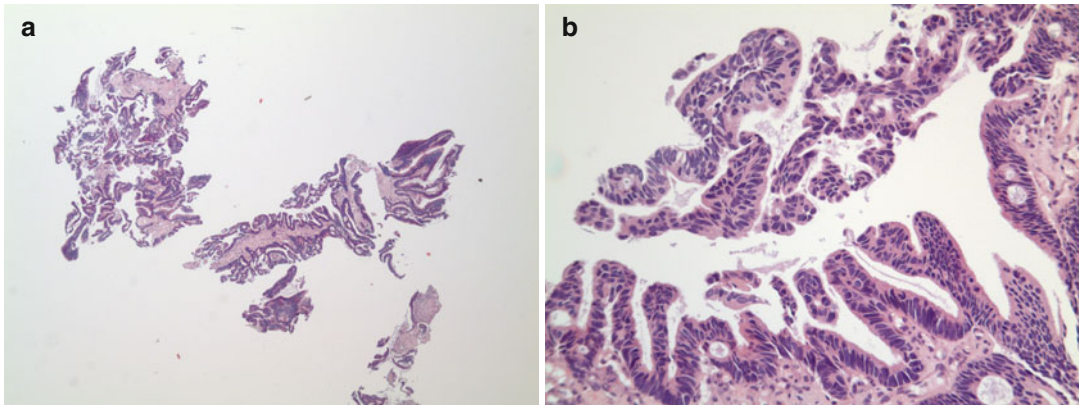

Fig. 5.10 (**a**) Transvaginal biopsy of ovarian mass. This patient had high grade CGIN with foci of adenocarcinoma diagnosed 6 years previously. The features are of metastatic adenocarcinoma of endocervical type (low power). (**b**) High-power view of the tumour

Comparison with the previous cervical lesion will resolve this issue. Diffuse strong nuclear positivity for p16 supports a metastasis from the cervix as most of the usual types of cervical adenocarcinoma are HPV related. In situ hybridization for HPV DNA is a more specific test and would provide definitive evidence of metastasis from a cervical adenocarcinoma.

Metastases from other organ systems are encountered less frequently on needle core biopsies, and the reader is referred to Chap. 16 for a comprehensive review of the subject.

Problems in the Interpretation of the Core Biopsy

The core biopsies have the advantage over cytological preparations in that they enable the tissue architecture to be preserved in addition to the cytology facilitating accurate diagnosis together with typing and grading of the tumor. This latter feature has become of importance for the inclusion in clinical trials. In the setting of an MDT core biopsies are an excellent method for a rapid tissue diagnosis. On occasion the material may be suboptimal for diagnosis. The tumor may have been missed on biopsy. Sometimes the tissue shows inflammatory changes which might suggest that there is a neoplasm adjacent to the site of biopsy. Of course there are rare occasions when the clinical and radiological appearances would have led the team to believe that it was a malignant process that was being investigated, but it turns out to be an inflammatory process. It is worth examining the biopsy tissue at multiple levels before labeling it as nondiagnostic as, on occasion, the examination of levels has revealed carcinoma in further deeper sections. Sampling can be an issue, as the biopsy may not be representative. This can occur with a biphasic tumor where only one component is represented on the biopsy, carcinosarcoma with the carcinomatous component represented on the biopsy, or a sarcomatous component identified with heterologous elements in a tumor that turned out to be an adenosarcoma with sarcomatous overgrowth on the resection specimen.

On rare occasions, it is not possible to characterize a tumor further than an undifferentiated carcinoma despite the use of immunohistochemical markers a primary site of origin cannot be suggested. However, other tumors that enter into the differential diagnosis must be excluded such as a lymphoma, sarcoma, and malignant melanoma using known immunohistochemical markers.

Learning Points

- IGCB is a useful method of obtaining a tissue diagnosis.
- It must be used in an MDTM setting with appropriate clinicopathological correlation.

- The vast majority of cases are high-grade serous carcinoma.
- Immunohistochemistry is required to exclude the differential diagnoses.
- In patients with a previous breast carcinoma, the likelihood is a Mullerian primary carcinoma rather than recurrent breast carcinoma.

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Frozen Section Use in the Diagnosis of Ovarian Pathology

6

Paul A. Cross

Abstract

This chapter aims to highlight the pros and cons of the use of a frozen section (FS) in the diagnosis of ovarian pathology at the time of surgery. Like all approaches, it is not perfect and does rely on a very close working relationship between the pathologist and surgeon to ensure that when, and if, it is used the maximum patient benefit is obtained. A FS (like all diagnostic pathology) will never be fully accurate. However, if one accepts its potential limitations, it can be a very good tool in the management of patients with suspected ovarian pathology. This chapter will aim to highlight major issues with the use of a FS service for ovarian diagnosis and will outline potential pitfalls. It is not intended to be a textbook of FS ovarian pathology – the remainder of this book describes in far greater detail the diagnostic criteria for ovarian pathology diagnosis. These still apply even if based on FS material. A recent survey has shown that FS use in the UK, when performed, is largely done for ovarian/pelvic masses and lymph nodes in cervical cancer surgery.

Introduction

This chapter aims to highlight the pros and cons of the use of a frozen section (FS) in the diagnosis of ovarian pathology at the time of surgery. Like all approaches, it is not perfect and does rely on a very close working relationship between the pathologist and surgeon to ensure that when, and if, it is used the maximum patient benefit is obtained. A FS (like all diagnostic pathology) will never be fully accurate. However, if one accepts its potential limitations, it can be a very good tool in the management of patients with suspected ovarian pathology. This chapter will aim to highlight major issues with the use of a FS service for ovarian diagnosis and will outline potential pitfalls. It is not intended to be a textbook of FS ovarian pathology – the remainder of this book describes in far greater detail the diagnostic criteria for ovarian pathology diagnosis. These still apply even if based on FS material. A recent survey has shown that FS use in

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the UK, when performed, is largely done for ovarian/pelvic masses and lymph nodes in cervical cancer surgery [1].

Why Do a Frozen Section?

The management of ovarian pathology is impossible without tissue to make a diagnosis. The ovaries can be the site of a vast range of pathologies, from benign to malignant, with the added problem of borderline lesions, but can also be the presenting site of malignancy elsewhere as a secondary deposit(s). For this reason, the surgeon/oncologist cannot base any rational treatment option until a definite diagnosis is arrived at, to both benign and malignant but also to primary site (if possible). Given this, the ideal would be preoperative diagnosis, but this is not always possible. If there is a cytology sample (e.g., ascitic fluid, FNA), then this may yield a diagnosis (see Chap. 7). However, this can often be inconclusive or at worst misleading [2], and a histology sample will always remain the most likely sample to yield a definitive diagnosis. It is also important to decide in what circumstances a FS will be used at operation. Is it for diagnosis of malignancy (irrespective of stage), or is it only for cases of potential early-stage disease? One protocol can only do a FS for potential stage 1 or 2 disease, and hence a staging procedure may be required, as opposed to a higher-stage disease where debulking surgery rather than staging is performed. It can be argued that these later higher-stage cases are equally in need of diagnosis. However, the surgical approach is very different between the two.

Often much liked by surgeons, a FS can be viewed with suspicion by pathologists. A FS can be stressful for all involved and does require guaranteed availability of laboratory staff and a pathologist. There is also a large difference between an occasional "one off" FS and a routine FS service as is used in some centers for intraoperative ovarian diagnosis.

In clinical terms a FS offers the possibility of a one-stage operation, rather than the more usual biopsy-action flow (e.g., operate, take biopsy, await report, and then reoperate some time later to perform definitive surgery). This is more akin to the "see and treat" vs. "see and biopsy" approach used in colposcopy in which gynecological pathologists and surgeons are familiar with. The one-stage FS approach can reduce the need for a second operation and anesthetic, and their inherent potential risks, but *only* if the FS diagnosis is robust enough to ensure that correct surgery is undertaken with a high probability of success [2, 3].

A FS can also allow for tissue taken at one hospital site to be remotely reported at another hospital site if the correct technology and skills are available [4]. This of course applies to all histology in principle, but given the need for rapid diagnosis, the expertise to report a FS (given its immediate patient management impact) does not have to be at the same site as the actual surgery.

Given that fresh tissue will be sent to the laboratory for the FS to be taken, a FS service also allows fresh tissue to be taken for research, given of course that the correct patient consent and research/ethics protocols are followed.

Ground Rules for FS Use

Before any FS is undertaken, both sides (surgeon and pathologist) must understand the use and issues with a FS. A FS will not always yield a definite diagnosis, but this should be relatively rare. Both sides should accept and understand this. It is imperative that an agreed protocol is decided upon before an ovarian FS is undertaken, particularly with respect to cases called borderline on FS. If a FS is not going to affect what procedure the surgeon actually performs at that time, then a FS is not needed – this should go without saying but does need emphasizing!

In reporting terms a FS diagnosis has one of three outcomes: benign, malignant, or defer to paraffin (relatively uncommon). Very few papers quote a "defer" rate, but rates of zero [5] to up to 6.3 % [6] are quoted in studies with reasonable numbers of cases. However, in ovarian pathology there is also the category of borderline (BL). This must be accounted for in any agreed protocol

between pathologist and surgeon. This category is certainly not as robust a diagnosis as one of benign or malignant and the degree of inaccuracy does appear to depend largely on the size/weight and type of lesion [5, 7] and to some extent on the experience of the reporting pathologist [8]. Our protocol places a BL lesion into the malignant category clinically and would evoke a staging procedure. However not all would agree and feel this could lead to unnecessary overstaging [9]. It must be clear how a BL diagnosis will be responded to in the clinical protocol. A diagnosis of "at least borderline" is perfectly acceptable in difficult BL/malignant cases [10] as it does aid the surgeon.

FS Booking/Transport

Given that nearly all cases of suspected malignancy will be discussed within the setting of an MDT preoperatively, cases where a FS may be of use can be identified. This allows the surgeons to plan their operating lists and equally allows them to prewarn the laboratory of the need for a FS ideally at least 24 hours in advance. This allows the laboratory to ensure sufficient staffing, including alerting the pathologist. How such a planned FS is dealt with and rostered within the laboratory will depend on each individual laboratory's approach to how it works. It is vital to ensure a reporting pathologist is identified. Given that a FS will only involve up to 5–10 min of a pathologist's time (description/block taking/reporting), this should not be overly disruptive to the pathologist's routine working pattern. Such prior booking also ensures that all staff involved with the specimen transfer (in particular portering staff and lab reception staff) are alerted in advance.

If the FS is no longer required for any reason, it should also be self-evident that the surgical team should inform the laboratory so that they can be "stood down" for that FS.

It is obvious that a reliable cryostat is essential to the performance of a FS. Most laboratories within the UK will have one, but it is often infrequently used. In the UK, FS use is not common, whereas in other countries, e.g., the USA, it is far more used. Hence, in a UK laboratory the general experience of the FS process is far more limited, both at the technical and pathologist level. From the technical point of view, it may require practice to ensure consistent, quality sections. This must cover not only the freezing/section stage but also hand staining.

How Long Does a FS Take?

Given that the patient is anesthetized, the time of a FS must not materially impact on patient safety in the operating room. One must factor in the total time for the FS process – from theater, transport to the laboratory, processing, and reporting. For FS overall, Novis and Zarbo [11] found that over 90 % of FS can be processed and reported back to the surgeon within 20 min of receipt within the laboratory. This is a target that is achievable. Mean times of 18.5 min, range 10–45 min, are reported [12], with the longer times usually reflecting FS being taken on more than one ovary or site.

Communication of Results

Whenever a FS is performed, clear, concise, unambiguous communication is vital between the requesting surgeon and reporting pathologist. It should go without saying that the patient demographics on the request form and specimen pot must be consistent with each other and easy to read. The request form must state why the FS is required and also any relevant clinical findings or history. This should include operative findings, but also any abnormal tumor markers. It must also include any relevant medical history, and in this context any previous history of malignancy is essential. It is amazing how often such a history appears to be forgotten about! Given the potential for secondary malignancy presenting as an ovarian mass, any history of prior malignancy is vital for the reporting pathologist. Such information may allow the pathologist to suggest that any malignancy may not be of ovarian origin and

hence point the surgeon in another direction. However, it must also be remembered that this may be the first presentation of an occult primary malignancy elsewhere, and in this context the separation (if possible) of a secondary malignancy from an ovarian primary can dramatically affect the surgeon's approach.

It is also vital that the form indicates who the pathologist must contact to give the FS report. Invariably this will be by telephone. Very few (if any) operating theaters these days have the FS taken and processed within the theater suite, and most specimens are conveyed by portering staff to the pathology laboratory for processing. It is all too easy to waste 5–10 min ringing around to find the correct operating theater or surgeon in order to give back a report!

Given that any FS is an urgent request, it must not be delayed in its transport or reporting. The use of a high visibility FS form, e.g., yellow/orange, can help ensure that LEAN visual management principles are applied [13].

Health and Safety Aspects

A FS is a fresh unfixed specimen and as such is a potential health risk to anyone who may come into direct contact with it. The specimen must be transported in compliance with appropriate guidance and is suitably identified as a fresh specimen. Any standard operating procedure (SOP) must cover aspects of potential spillage/leakage, as well as handling for dissection purposes to minimize any possible unnecessary exposure. If the patient is of potential high-risk category (e.g., hepatitis B positive, HIV positive), then consideration should be given as to whether the FS could be avoided and routine histology performed to minimize any possible exposure risks.

Gross Description/Block Selection

The gross description of the FS sample is just as vital as the actual histological reporting. In general the FS will be a salpingo-oophorectomy but depending on the operative findings may be an oophorectomy, an ovarian biopsy, or just a "pelvic mass." Occasionally a TAH/TLH with BSO may be received. Whatever specimen is received, the general principles of macroscopic description apply [14]. Particular note should be made of any defect/tear of any ovarian mass and as to whether it is intact or not and if any obvious tumor is seen on the ovarian surface or elsewhere. Weighing the intact ovarian mass is advised for future reference. On section, mention must be made of the nature of the ovarian lesion, i.e., solid, cystic, or solid/cystic (Figs. 6.1 and 6.2). Is it obviously necrotic or congested/hemorrhagic? If cystic, is it uni- or multilocular? Do any cysts contain serous-type fluid, mucin, blood (fresh or clotted), or a combination? Any obvious coloration may also point to a potential lesion (e.g., some sex cord-stromal hormone-producing lesions may have a yellowish tinge). A full description, however, can

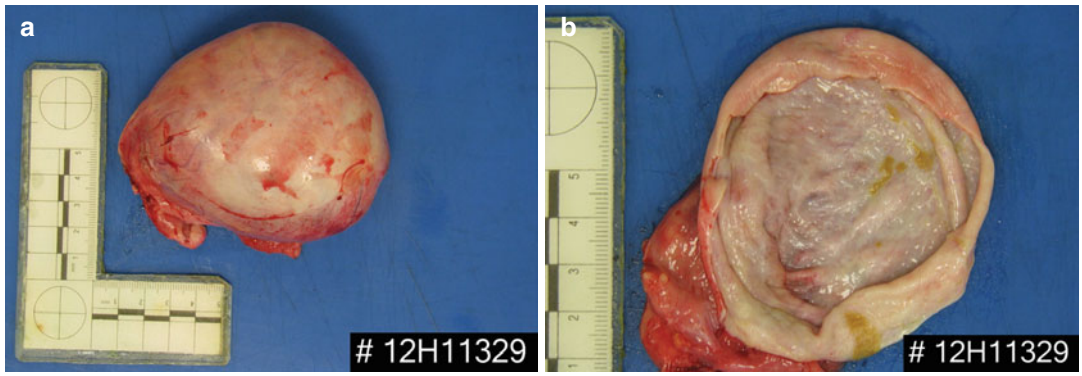

Fig. 6.1 Gross view of intact simple serous cyst (**a**) and of smooth, internal lining (**b**)

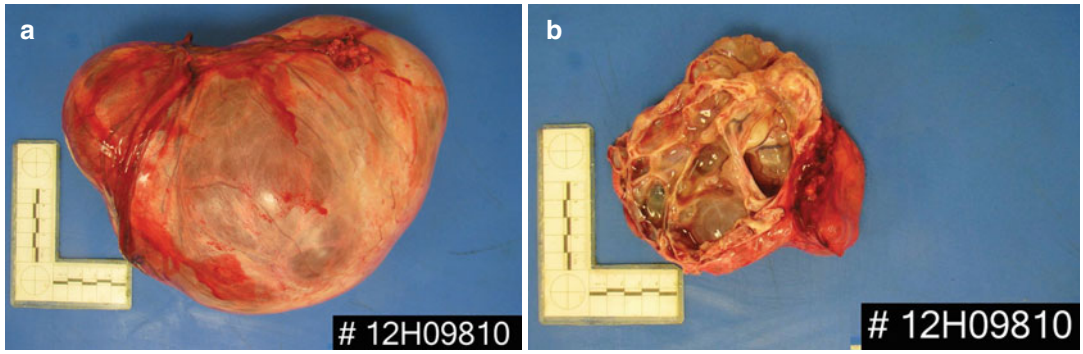

Fig. 6.2 Gross view of simple mucinous cyst (**a**) and of multiloculated appearances on section (**b**)

await until after proper fixation and until routine blocks are taken.

For a FS any tissue taken must try to be representative of the lesion overall and be reported upon. Any necrotic areas must be avoided, as must any obviously calcified foci, as they will not process for a FS. If the lesion is solid, blocks from apparently non-necrotic areas are advised. If cystic, then any firm/raised/locule intersections are recommended, as these appear more often as the site of any possible atypia if present. If purely cystic, then a "Swiss roll" approach can be used to maximize the amount of cyst wall tissue taken. If the whole lesion is apparently necrotic/infarcted (and invariably congested), then the least affected areas are advised. However, in the latter circumstances, one may anticipate that the FS may not be of diagnostic use.

How Many Blocks?

Any FS is a trade-off between obtaining a report that represents the ovarian lesion and getting the answer to the surgeon in a timely manner without compromising patient safety. In my experience, two blocks for a FS allows for this balance. Careful block selection (as outlined above) maximizes the potential diagnostic yield. It also allows a backup section if for some reason the other is problematic to process or interpret. The use of two blocks allows a turnaround time of typically 15–20 min from receipt in the laboratory to report issue. This time is not wasted by the surgeon, often allowing them to proceed with some of the operation that they need to perform (e.g., hysterectomy). More blocks can be taken if desired, but the time taken will be longer. It is likely that if more FS blocks are taken, the diagnostic accuracy may improve, but there is no apparent evidence in the literature for this despite the intuitive nature of this statement. Less blocks may be quicker, but diagnostic accuracy again may be less with less material to review.

FS Appearance/Artifacts

Because the FS tissue is not processed through the usual histological process of dehydration/rehydration, a FS has a different, albeit similar, microscopic appearance to routinely processed histological material. While the architecture is essentially the same, the cellular detail is different, with larger nuclei and often more prominent nucleoli – i.e., less shrinkage artifact. Mitotic figures, whatever the nature of the lesion, are often more apparent than on routine material. Overinterpretation of what are routine FS changes must be avoided, and comparison with any recognizable benign tissue within the section should be taken to assess variation from the norm. Avoidance of any necrotic/infarcted areas is essential, as is overinterpretation of pyknosis/karyorrhexis. Hopefully careful block selection as outlined above will minimize this. There is also often an apparent multilayering of simple epithelium more so than is seen in routine

processed sections. This is most likely due to the FS being thicker than the routinely processed sections, typically of the order of 6–7 um rather than the routine paraffin-processed tissue section of some 3 um. The ability to see fine histological detail in a FS is also less, and more reliance on disorganized/abnormal architecture, malignant-type necrosis, nuclear pleomorphism, atypia, and abnormal/excessive mitotic activity are key, but are still easily recognizable. Specific issues with specific lesions will be dealt with later on.

Pathologists sometimes balk at reporting FS due to a lack of familiarity with the material and FS appearance. This is understandable, but experience can easily be obtained. In the initial stages, samples can be sent fresh to the laboratory and FS material taken but not reported upon urgently. Pathologist experience and confidence will soon develop if this is done. FS material from other centers can also be used to gain experience. Correlation of the FS slide with the routinely processed equivalent block is essential to educate the reporting pathologist to be able to better recognize features for future reporting.

How Good Is a FS?

There is a wealth of literature on the use of FS in the reporting of ovarian pathology. Many are small series, dealing with a general service, but many lack sufficient detail to allow more in-depth analysis. Several reviews [6, 15, 16] highlight studies that do allow meaningful data analysis, as do some of the larger single-site papers [5, 12]. Heatley [15] makes the plea for papers detailing a FS service to include more detail on tumor types, but also a more detailed breakdown by report (benign, malignant, and also borderline) and also of clinical context/picture. Overall, the outcomes for a FS do vary by tumor type (including size and weight) and diagnostic category. Table 6.1 outlines overall values and suggests that ovarian FS can be an effective, accurate tool. One may ask what the equivalent values are for the "gold standard" of routinely processed histological material, to which the FS is of course compared. FS reporting compares very favorably considering the limitations of less blocks and a more pressurized reporting situation.

How a FS is evaluated compared to the paraffin-processed "gold standard" is also a moot point. While most would quote sensitivity and specificity, values for positive predictive value (PPV) and negative predictive value (NPV) are quoted [15], as are values for pre- and post-test probability [12] as well as likelihood ratios [15, 16]. These different approaches all show good operating values for ovarian FS. The poorest value in any of these approaches is with a borderline FS diagnosis. How the borderline category is grouped (with benign or malignant) also alters the overall values. Table 6.2 gives a flavor of these other approaches.

The paper by Cross et al. [12] summarizes the overall data from their center as follows in

Table 6.1 Pooled typical literature values for sensitivity and specificity for ovarian FS diagnosis by report category

	Benign vs. BL/carcinoma		Benign vs. BL		Benign vs. carcinoma		BL vs. carcinoma	
	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)	Sensitivity (%)	Specificity (%)
Medeiros et al.[a]	99	88	99	66	94	99	91	95
Heatley[a]	98.7	89.9	98.9	70.8	99.7	95.8	93.6	93.1
Geomni et al.[a]	65–97	97–100	–	–	71–100	99.3–100	–	–
Ilvan et al.[b]	100	94.9	100	86.8	100	97.2	100	88.9
Cross et al.[b]	98.6	91.2	99	72.3	99.6	95.6	98	83.5

Some values derived from values within papers

BL borderline tumor

[a]Literature review based on suitable papers (Medeiros et al., 14 papers, 3,659 women; Heatley 18 papers, 4,542 women; Geomoni et al., 18 papers, 4,387 women)

[b]Large single-center studies (Cross et al., 1,342 women; Ilvan et al., 617 women)

Table 6.2 Typical literature values for other statistical approaches at looking at FS diagnoses

	Benign vs. carcinoma	Benign vs. BL	BL vs. carcinoma
PPV	97.9 (97.3–98.4)	94.9 (94.1–95.7)	77.7 (73.5–87.4)
NPV	99.5 (98.9–99.7)	92.3 (89–94.6)	98.2 (97.3–98.8)
LR+	23.99 (18.52–31.1)	3.384 (2.94–3.89)	13.293 (10.94–16.15)
LR−	0.003 (0.01–0.006)	0.015 (0.01–0.022)	0.069 (0.047–0.103)

From Table 2 in Heatley [15]
BL borderline, *PPV* positive predictive value, *NPV* negative predictive value, *LR* positive likelihood ratio, *LR* negative likelihood ratio

clinical terms: of the 1,342 women who had a total of 1,439 FS, 1,268 (94.5 %) women had the correct surgical procedure at the time the FS was reported; 58 (4.3 %) women were understaged, and 16 women (1.2 %) were overstaged based on the FS report. However, the actual effect of understaging may be clinically minimal [17].

The literature indicates that certain types of ovarian tumors are less accurately reported at FS than others. This applies in particular to mucinous lesions [5, 12, 18]. These are invariably larger, heavier complex lesions than other epithelial lesions. The often focal nature of both borderline and malignant changes in mucinous tumors is also an issue. Current evidence also suggests that the majority of mucinous lesions of the ovary are not of primary ovarian origin, and hence any mucinous lesion should be considered potentially of metastatic origin [19], even if apparently of "obvious" benign appearance. Given this, any mucinous tumor should be considered potentially malignant and may require close surgical examination of the bowel and appendix in particular.

The diagnosis of BL on an ovarian FS is less reliable than one of benign or malignant. The issue depends on how a BL lesion is classified and hence the clinical action resulting from that diagnosis (staging or not). Accuracy (i.e., a BL diagnosis remaining as a BL diagnosis) is of the order of 50 % [12, 16]. The majority of the incorrect diagnoses uplift a BL diagnosis to one of malignancy; hence, a clinical approach which places a BL diagnosis along with a malignant diagnosis is tenable and does not have a real clinical impact [17]. This does vary by epithelial subtype and is least accurate with a mucinous lesion [12, 18, 20, 21]. These papers, and the literature review ones, show that increasing tumor size (and weight) renders the BL diagnosis less robust. In this situation, a variation from 91.7 % accuracy for small (<450 g) lesions to 66.7 % for larger (>1,360 g) lesions has been found [21]. The false-negative reporting rate (i.e., called BL but actually carcinoma) can vary 5.4-fold between serous BL and mucinous BL [12], reflecting the greater diagnostic problems with a mucinous lesion.

Errors with FS diagnosis essentially are due to two causes: sampling errors or interpretational errors [12]. The former may reflect poor block selection, but is intrinsic to the FS approach where only limited material is taken due to time constraints. Interpretational errors are also inherent to diagnostic pathology. Educational review of such cases by the reporting pathologist team can help reduce this, but this should be common practice in all cellular pathology departments to help reduce inaccurate diagnoses.

The overall outcome of FS reporting must be of sufficient quality to allow the surgeon who is relying upon it to base their surgical action upon it. This information is also a necessity in ensuring that the patient herself has accurate information to make an informed decision for operative consent. This is vital, as effectively the woman does not know exactly what procedure she will be having before surgery, and hence she also must have an appreciation of how good (or bad) a FS can be upon which her exact surgery is going to be based. Each unit should produce its own data on this with sufficient numbers and time, but initially typical literature values can be used to help with these discussions.

Specific Diagnostic Challenges

The criteria for diagnosis in a FS are essentially no different to those of a paraffin section, although the appearances are different from that

of the routinely processed material as outlined above. It is not intended to describe all the types of lesions or tumors that may be seen within an ovary, which are covered in more detail in the rest of this book. However, certain problematic areas will be outlined to help with FS diagnosis.

Benign Nonneoplastic Lesions

It must not be forgotten that an ovary (or ovaries) can appear clinically suspicious or even malignant without actually being so. The literature does not cover this aspect of ovarian FS well, being not surprisingly preoccupied with malignant diagnoses. Table 6.3 outlines the typical nonneoplastic diagnoses quoted at ovarian FS. Processes such as endometriosis (Fig. 6.3), salpingo-oophoritis, or physiological cysts can present as unilateral (or sometimes bilateral) masses, sometimes associated with torsion. The FS of viable areas will show typical features of these processes, and critical evaluation of the lack of malignant features ought to allow correct diagnosis. Many ovarian endometriotic cysts may appear fibrotic, with evidence of old hemorrhage, and the classic features of both benign endometriotic stroma and glands may not always be apparent.

Table 6.3 Benign nonneoplastic diagnoses at ovarian FS

Nonneoplastic diagnosis	Cross et al. (11.6 % of all FS diagnoses)	Ilvan et al. (18.3 % of all FS diagnoses)
Endometriosis	112	71
Inflammation/abscess	37	13
Normal ovarian cysts	18	27
Others	0	2
Total numbers	167	113

Features of congestion and hemorrhagic infarction may be seen in any ovarian lesion – these appearances typify torsion – and inspection of viable areas (if any can be found) is essential to try and allow for diagnosis. The gross appearance is typically that of a markedly congested and necrotic mass, the nature (solid or cystic) of which will reflect the underlying lesion. In many torted ovaries, however, residual viable tissue can be very problematic to find and may not be identified even after extensive sampling for routine processing, and so FS material may not yield a firm diagnosis in this situation.

A hydrosalpinx may be mistaken for an ovarian cyst and submitted for FS as an ovarian/paraovarian or tubo-ovarian cyst. It does not matter for the purposes of a FS as all are essentially those of a benign serous cyst, the exact origin of which can await more extensive sampling on routine processing. Features such as the ovarian cortex or primordial follicles may help identify the ovary, but are not essential if no malignant features are seen.

Physiological cysts (e.g., follicular or luteal) (Fig. 6.4) are unilocular, smooth, and invariably

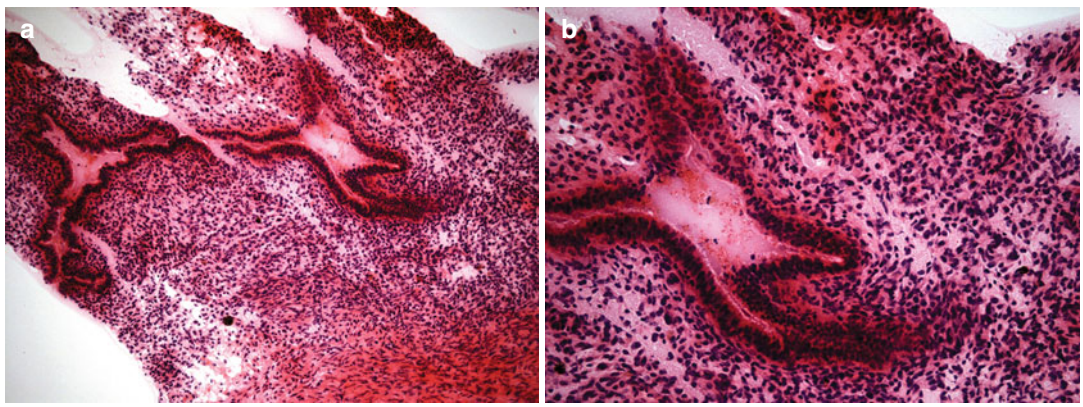

Fig. 6.3 Frozen section appearance of benign endometriosis showing glands and stroma (**a** F×10), and at higher view (**b** F×40), note the bland glands and stroma and intimate association of the two

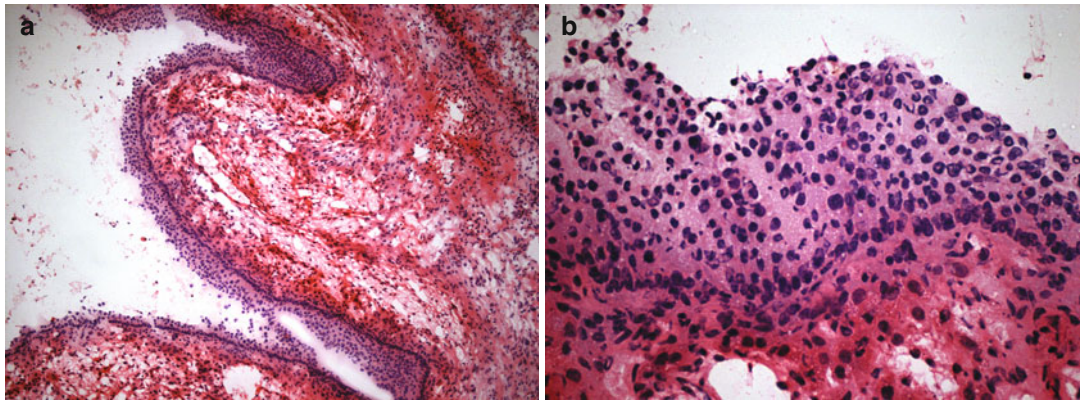

Fig. 6.4 Simple follicular cyst (**a** FS×10, **b** FS×40). Note multilayering and variable nuclear size. An occasional mitosis may also be seen. However, note the simple cyst architecture, and no invasion is present

solitary except under unusual circumstances (e.g., hyperstimulation, polycystic ovary syndrome) but again show features of benign cysts. Exact classification is not essential at a FS, but be aware that physiological cysts can show quite a high mitotic rate, and some luteal cysts can show some pleomorphism, but again the overall architecture, and invariably history, will help in diagnosis.

Specific Problem Areas in Ovarian FS Diagnosis

Primary Ovarian Epithelial Lesions

The majority of ovarian lesions in adults subjected to a FS will be of epithelial origin. Many will be simple/benign cysts. These are not usually problematic. For more detailed descriptions of ovarian pathology in general, the reader is advised to read the other chapters of this book. While this section will not cover every possible ovarian pathology, it will from experience try and highlight common problems.

Serous cysts are usually unilocular and smooth lined (Fig. 6.5). Serous cysts may have papillary areas on the internal aspect, but these can also be encountered on the capsular surface as exophytic ovarian lesions (Fig. 6.6). These papillary (or solid) areas, if present, are the ones best sampled. Borderline serous lesions may be architecturally complex with multilayering and show some mild cytological atypia (Fig. 6.7). Such low-grade borderline areas and true micropapillary lesions may require careful scrutiny under the microscope, but the degree of architectural complexity seen together with the minor cytological changes is too marked for simple benign serous epithelium. High-grade serous borderline change is not problematic as marked nuclear atypia with epithelial stratification and a high mitotic rate are present. These features usually accompany architectural complexity (Fig. 6.8).

Mucinous lesions are usually multiloculated complex cysts with multiple cyst intersections and often solid areas (Fig. 6.2). Again, solid/cyst intersection areas are best sampled for FS. Any diagnosis of a mucinous lesion (even if apparently benign) (Fig. 6.9) may reflect secondary spread [19]. If apparently benign this appears to be at low risk, especially if the lining is endocervical in type. If enteric and BL, then it is more difficult to be so certain (Fig. 6.10). Given this problem, an algorithm based on size and uni- or bilaterality of mucinous lesions [22] has been proposed to help classify malignant mucinous lesions as primary or secondary. In essence, this classifies all bilateral mucinous carcinomas as metastatic, unilateral mucinous carcinomas <10 cm as metastatic, and unilateral mucinous carcinomas ≥10 cm as primary ovarian mucinous carcinomas and can correctly classify up to 90 % of neoplasms. However, while this may be of

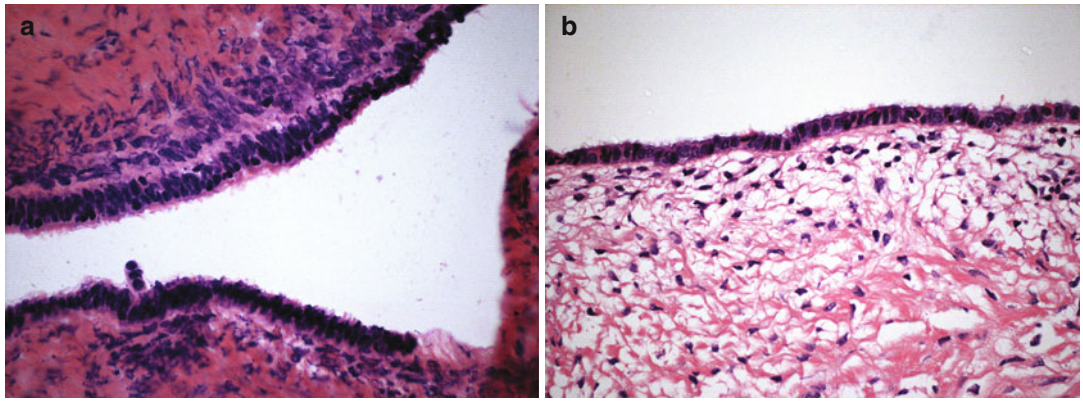

Fig. 6.5 Simple serous cyst (**a** frozen section ×40, **b** paraffin section ×40). Note apparent multilayering on FS but lack of other atypical features, confirmed on the PS

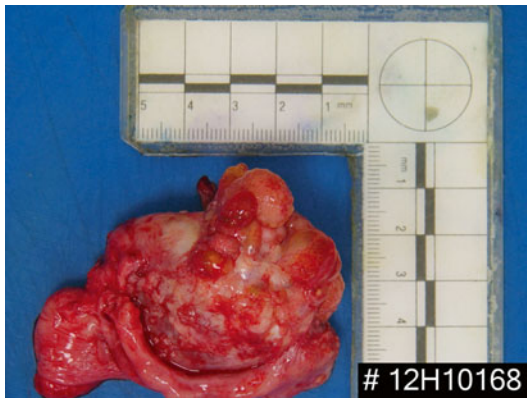

Fig. 6.6 Gross specimen exhibiting surface papillary areas, in keeping with serous surface proliferation. It is not possible to say if this is benign or not on gross inspection

assistance, it does require information on both ovaries (or both for FS evaluation) and also has an error rate. Others [23, 24] have suggested amendments to this approach with varying degrees of success.

Secondary mucinous tumors are most commonly of the large bowel (Fig. 6.11) or upper gastrointestinal tract, especially the stomach. One will often find the typical features of "dirty necrosis" or signet ring forms with these two particular sites, respectively, both of which can help identify and suggest secondary spread at FS. The appendix must also be considered as a potential primary site for mucinous lesions of the ovary. A FS which consists mostly (if not solely) of mucin may represent an inspissated mucinous cyst, a mucinous adenocarcinoma, or a pseudomyxoma. In these circumstances if only mucin is seen at FS, then a diagnosis as such cannot be given. In these cases given the inherent lower accuracy of a FS in the presence of a mucinous lesion, a diagnosis of borderline may be offered, and further surgery/staging may depend also on the overall clinical picture. If any epithelium is seen, this may point to one lesion or another and help with better classification.

Endometrioid adenocarcinomas are easily confused at FS with high-grade serous carcinoma, and this can also occur on routinely processed material. In practical terms it is not a distinction that will matter, as both will appear malignant, and exact classification can await routine slides. Clear cell carcinomas can be deceptively bland on FS (Fig. 6.12) – this may be due to the clear cytoplasm, but scrutiny of the nuclear features and overall architecture should help with diagnosis [25]. The distinction between primary and secondary clear cell lesions of the ovary is not possible on a FS. Carcinosarcomas can occur in the ovary but are invariably easily diagnosed as malignant given the typically high-grade malignant features, even if the actual mixed nature of the tumor is not recognized at FS.

Brenner (benign urothelial) tumors (Fig. 6.13) may present as tumors in their own right or be associated with mucinous tumors. Their typically irregular nests of cells may suggest malignancy

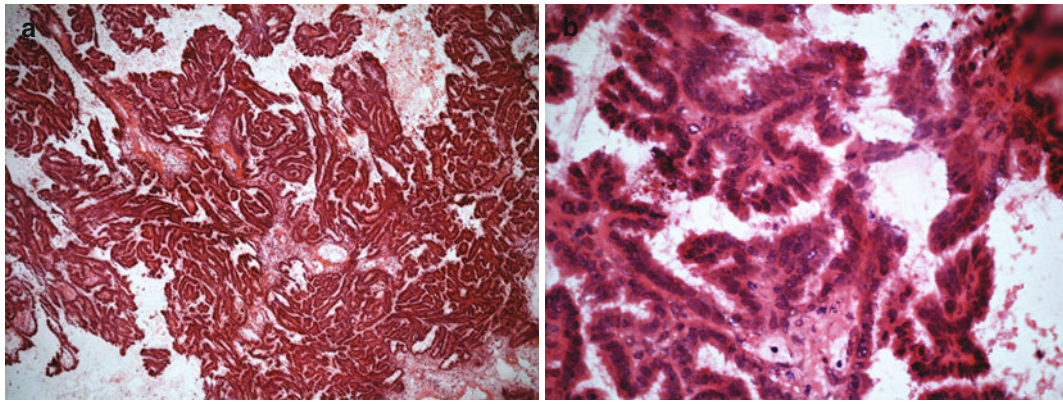

Fig. 6.7 Borderline serous lesion (**a** FS ×10, **b** FS ×40). Note architectural complexity and multilayering and nuclear variability

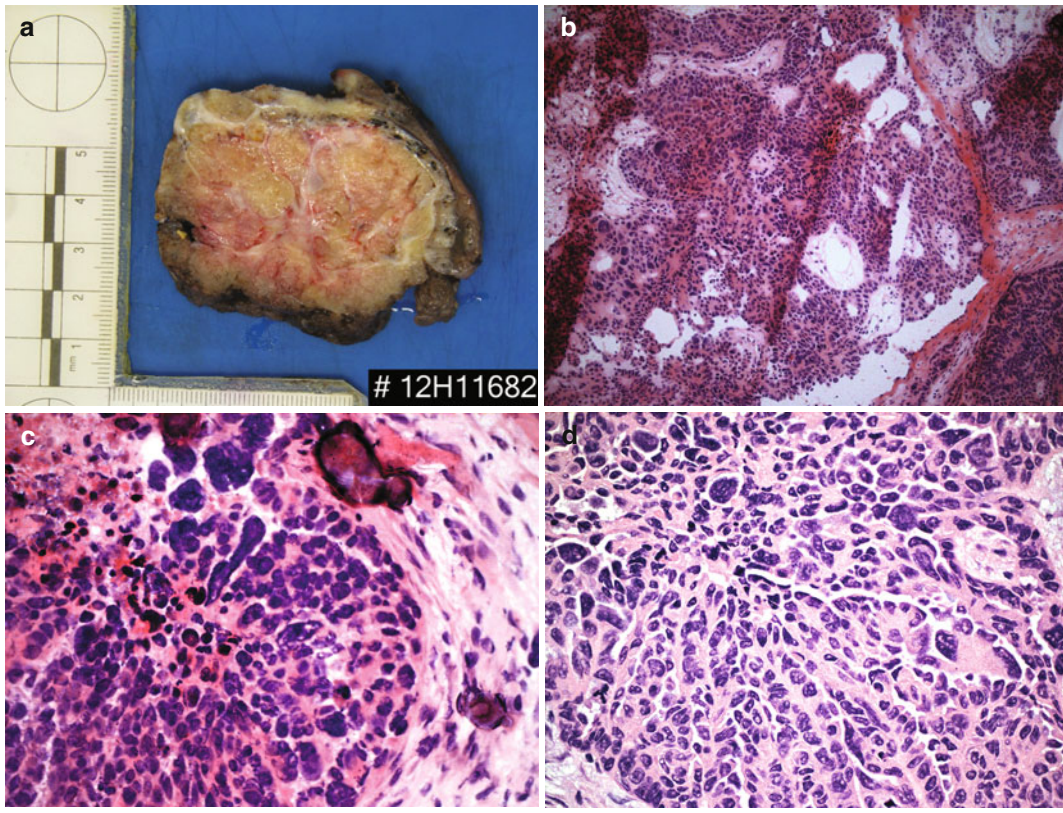

Fig. 6.8 Necrotic/solid ovarian tumor (**a**) which could be many lesions but is a high-grade serous carcinoma of the ovary. (**b**) (FS ×10) and (**c**) (FS ×40) show marked pleomorphism and mitotic activity, as well as some psammoma bodies in (**c**). PS of final histology from same block (**d**, ×40)

architecturally, but the typical urothelial nuclei with some nuclear grooves and lack of other malignant features should allow correct diagnosis. If necrosis, pleomorphism, or frequent mitotic figures are present, then a borderline or frankly malignant diagnosis should be considered, but such malignant urothelial lesions are relatively rare.

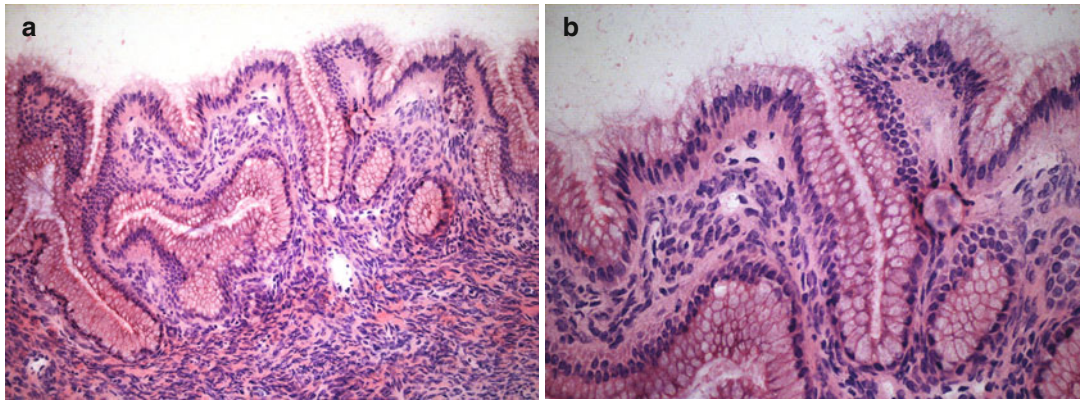

Fig. 6.9 Simple mucinous cyst. (**a**) (FS ×20) and (**b**) (FS ×40) show bland mostly enteric glands, with no atypical cellular features although some complexity of the actual cyst lining is not unusual

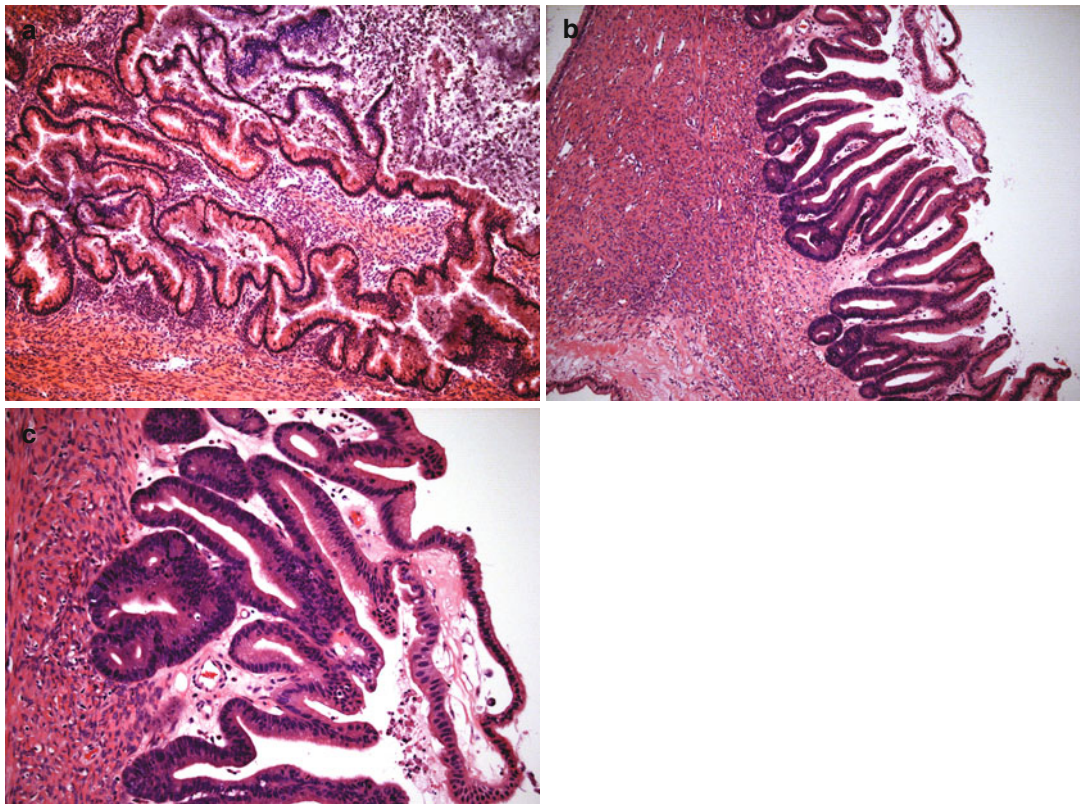

Fig. 6.10 Borderline mucinous cyst (all FS, **a** ×10, **b** ×10, **c** ×40) showing marked atypia and complexity, but with no invasion seen

Sex Cord-Stromal Lesions

The majority of sex cord-stromal tumors (SCST) will be benign, the most common being fibrothecomas. These can be cystic with associated Brenner tumors, but display no malignant features. However, one must look to exclude secondary tumors from the stomach (the classic

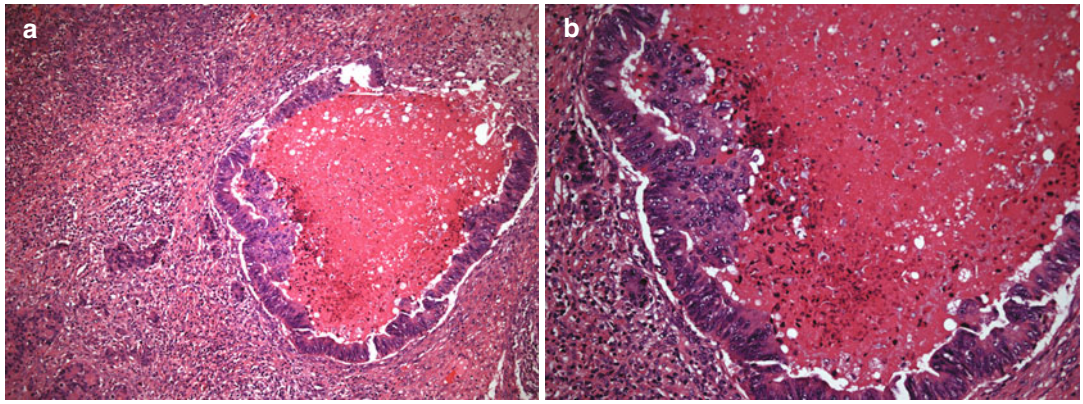

Fig. 6.11 FS of metastatic colonic adenocarcinoma (**a** ×10, **b** ×40). Note high-grade nuclear features and typical (when present) "dirty-type" necrosis

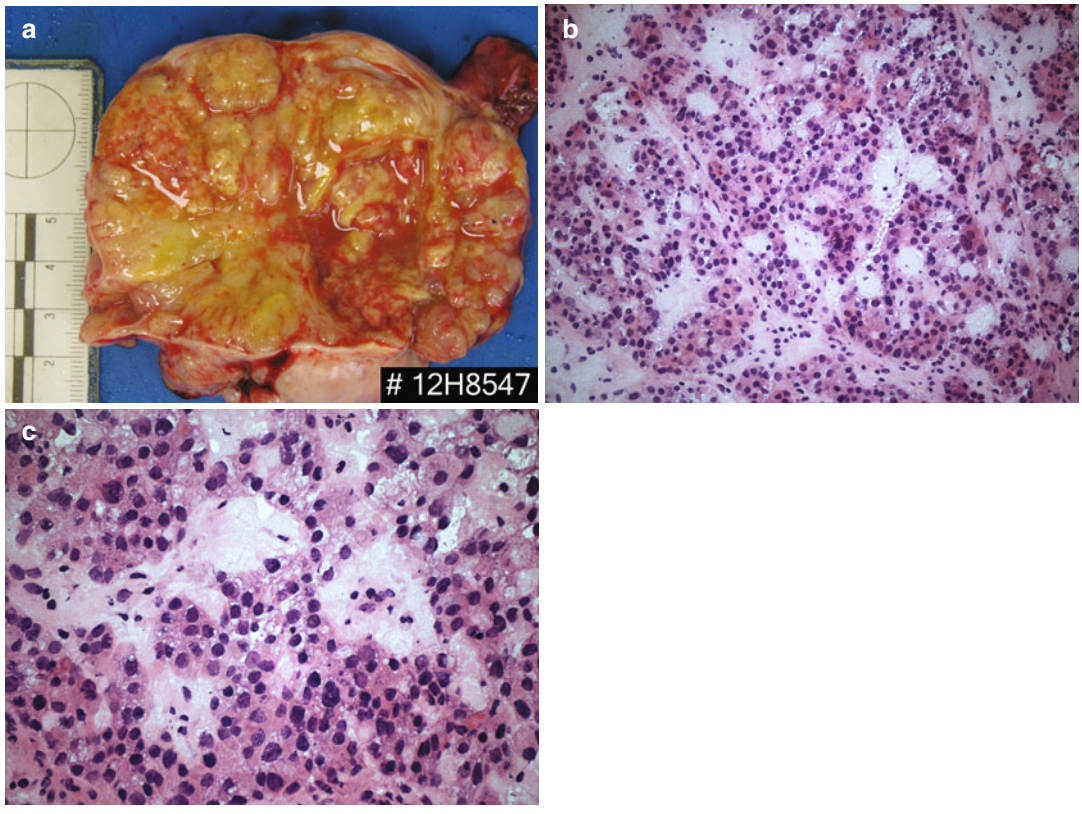

Fig. 6.12 Clear cell carcinoma. (**a**) Gross specimen with no obvious differentiating features from other malignant tumors. (**b**) (FS ×20) and (**c**) (FS ×40) show architecturally complex clear cells with markedly variable nuclei, but with very few mitoses typically. Focal necrosis is also present

Krukenberg tumor) or breast (especially lobular carcinoma) which can look grossly like a typical fibro-thecoma.

Tumors such as granulosa cell lesions (Fig. 6.14) or Sertoli-Leydig tumors will display their typical histological features. Both tumors

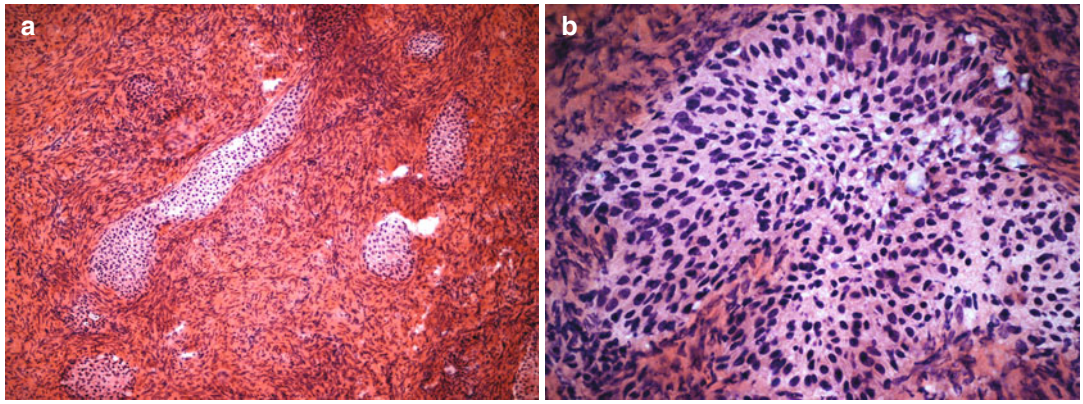

Fig. 6.13 Benign Brenner tumor (**a** FS ×10, **b** FS ×40). Some complex-appearing glands set in a fibrous stroma may at first glance appear alarming, but typical urothelial cells and nuclei and lack of other atypical features allow a correct diagnosis

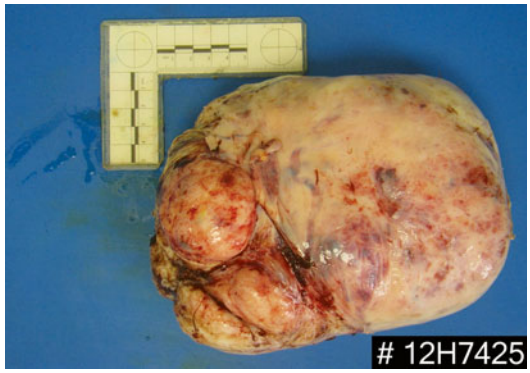

Fig. 6.14 Gross specimen of adult granulosa cell tumor

may display a wide range of appearances, but again the overall features make recognition of a non-benign process usually straightforward. A granulosa cell tumor may exhibit a yellow appearance to the naked eye, and while not diagnostic this may suggest a steroid-producing lesion.

A FS will not always allow exact classification as to the tumor type, but this is to be expected. The function of a FS is to assist the surgeon, and as such recognition of a non-benign process is key.

Germ Cell Lesions

The most common germ cell tumor (GCT) is the mature cystic teratoma (dermoid cyst). As such its gross appearance is diagnostic with a typical greasy hair ball centrally. Malignant change is uncommon and can be of epithelial origin (typically a squamous cell carcinoma) but can occur in any of the germ cell layers present. As such, sampling of solid/suspicious areas is advocated for FS, but one must be prepared to consider virtually any diagnosis possible. Monodermal teratomas (e.g., struma ovarii) can be more tricky (Fig. 6.15), but again are invariably associated with more typical teratomatous areas, but these may be lacking in a true monodermal teratoma. Secondary thyroid tumors to the ovary are rare.

Tumors such as dysgerminoma or embryonal carcinoma are straightforward on FS given their typical features.

Pediatric Ovarian FS

The range and type of possible ovarian pathology, both benign and malignant, are very different in the pediatric age range. No tumor has an absolute age predilection, but one must consider a potentially different range of tumors in the young. However, the approach to surgery is typically far more conservative in children, and diagnosis will usually rest on routinely processed material rather than on a FS diagnosis. As such, a routine FS approach to pediatric pathology in general appears more limited, which is supported

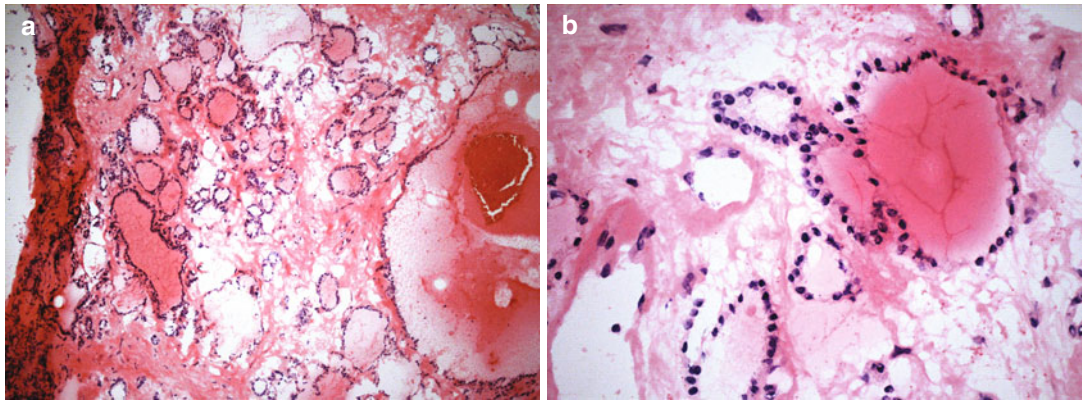

Fig. 6.15 Monodermal teratoma – struma ovarii (FS, **a** ×10 and **b** ×40). Note well-defined follicles with mostly single cell layers, with plentiful eosinophilic material (colloid)

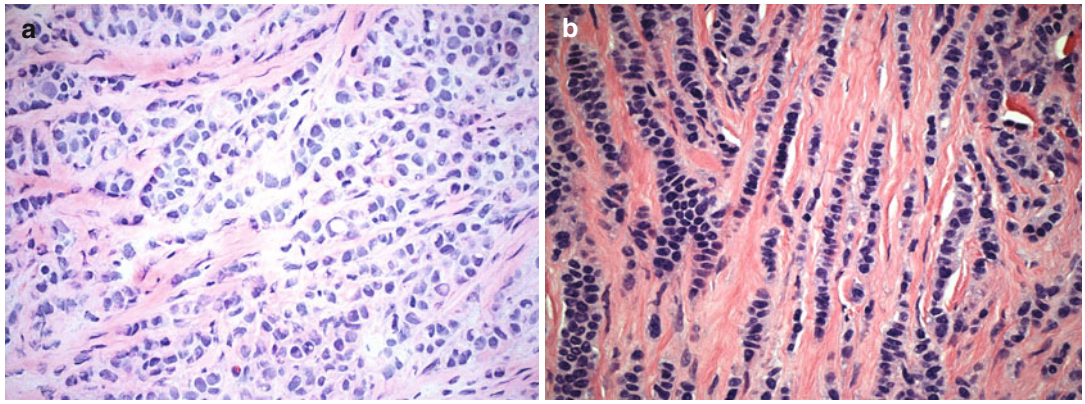

Fig. 6.16 Metastatic lobular carcinoma to ovary (**a** FS ×40, **b** PS ×40). Note typical lobular Indian file arrangement and occasional intracytoplasmic lumen in some cells

by a dearth of such literature on this topic [26], but it does highlight a relatively high error and defer rate.

Secondary Tumors to the Ovary

One of the questions that should be asked at every ovarian FS is "is the lesion seen primary ovarian or secondary to the ovary?" In the vast majority of times, it will be the former. However, as has been highlighted earlier, any mucinous lesion may be secondary. Tumors with a signet ring appearance are likely to be metastatic (possibly from the stomach or appendix), while ones with a neuroendocrine (carcinoid, well-differentiated neuroendocrine carcinoma) appearance can be primary or secondary. Colonic carcinoma has a typical histological appearance of overtly malignant adenocarcinoma with "dirty necrosis" which is not usually seen in other tumors (Fig. 6.11). Lobular carcinoma will have its typical appearance (Fig. 6.16), but may be difficult to spot (as may be signet ring cells) if scanty and set within a fibrous stroma. In many cases, however, identification as a secondary tumor may not be possible on a FS. Given that colonic carcinoma appears to be the most common tumor to spread to the ovaries, in many cases this can be identified. In one study, secondary tumors formed 4.8 % of ovarian tumors at FS, of which the largest single group were of gastrointestinal/appendiceal origin (57

out of 69) and overall 77 % were reported at FS as most likely of metastatic origin [12]. Such information, if it can be diagnosed, is of use allowing the surgeon to look for a primary site elsewhere and hence potentially adjust the surgical approach accordingly.

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7 The Role of Cytology in the Management of Ovarian Lesions

Sakinah A. Thiryayi and Durgesh N. Rana

Abstract

Cytology can serve as a useful tool in the diagnosis of both cystic and solid lesions of the ovary, in conjunction with radiological evaluation and multidisciplinary discussion. The use of peritoneal washings as part of the staging procedure for ovarian neoplasms is an important application of cytology. Sample types relevant to ovarian pathology may be divided into exfoliative specimens, including serous fluid samples (ascitic, peritoneal, and pleural), as well as peritoneal washings, and fine-needle aspirates (FNA).

Cytology can serve as a useful tool in the diagnosis of both cystic and solid lesions of the ovary, in conjunction with radiological evaluation and multidisciplinary discussion. The use of peritoneal washings as part of the staging procedure for ovarian neoplasms is an important application of cytology. Sample types relevant to ovarian pathology may be divided into exfoliative specimens, including serous fluid samples (ascitic, peritoneal, and pleural), as well as peritoneal washings, and fine-needle aspirates (FNA).

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Peritoneal Washing Cytology

The International Federation of Gynecologists and Obstetricians (FIGO) staging classification for ovarian cancer incorporates the results of peritoneal washing cytology [1], which constitutes part of optimal surgical staging of these tumors [2]. In addition, at the time of laparotomy, if fluid is present within the pelvis on entering the abdominal cavity, then this peritoneal fluid is submitted for cytological analysis. Washing of the peritoneal surfaces is typically undertaken by instillation of normal saline, with submission of the fluid for cytological assessment. The FIGO staging document recommends four washings, from the diaphragm, right and left abdomen, and pelvis [1]. In advanced disease with macroscopic evidence of disease outside of the pelvis (FIGO stages IIIB, IIIC, and IV), pelvic washings are not usually performed [3].

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As peritoneal washings are obtained during the surgical staging procedure, they are usually contaminated with blood, which may impair interpretation due to obscuring of cellular detail or dilution of tumor cells [4]. Preparation procedures undertaken in the laboratory aim to remove the red blood cells in order to produce an adequate, well-presented preparation for cytological interpretation [5]. The specimen may be prepared by various techniques to produce direct spread slides, cytospins, liquid-based cytology (LBC) preparations such as ThinPrep® and SurePath™, or a combination of these methods. The resulting slides may be stained by Romanowsky-type stains, such as May-Grünwald Giemsa (MGG), or by the Papanicolaou (Pap.) method.

The prevalence of the main subtypes of ovarian carcinoma influences the frequency with which each subtype is detected in peritoneal washing samples. Serous carcinoma constitutes 68–71 % of cases, clear cell carcinoma 12–13 %, endometrioid carcinoma 9–11 %, and mucinous carcinoma 3 %. In addition to being the most frequent tumor type, serous carcinoma constitutes the majority of cases (88 %) presenting at a late stage (III/IV) with more equal distribution of subtypes in early-stage (I/II) disease – serous 36 %, clear cell 26 %, and endometrioid 27 %. Although mucinous carcinomas represent only 8 % of early-stage disease, due to the lower prevalence of this subtype, this represents almost all of such cases [6].

Mesothelial Cells Versus Serous Borderline Tumors Versus Low-Grade Serous Carcinoma

The background cellular population of a peritoneal washing sample constitutes mesothelial cells, which may have numerous morphological appearances that are well described in standard cytology texts. It should be noted that in peritoneal washing samples in particular, as opposed to other serous fluid samples, the mesothelial cells may present as two apparently different cellular populations with flat, monolayered sheets of non-proliferating/resting cells and more 3-dimensional rounded groups of proliferating cells that may mimic tumor cells [7] (Fig. 7.1a–d). In addition, reactive changes may be seen in mesothelial cells in response to various conditions, including the presence of pelvic tumors, which may also result in diagnostic difficulty.

The presence of papillaroid fragments leads to the main diagnostic dilemma encountered in peritoneal washing samples, which is the distinction between reactive mesothelial cells, serous borderline tumors, and low-grade serous carcinoma. In addition, benign processes such as endosalpingiosis and endometriosis may also result in similar appearances and enter the differential diagnosis [8].

Reactive mesothelial hyperplasia results in tight clusters of cells with irregular edges that have low nuclear/cytoplasmic ratios, fine chromatin, and prominent nucleoli [9, 10] (Fig. 7.2a–c). Careful assessment of the cells within the groups may reveal features typically associated with mesothelial cells, such as intercellular windows due to microvillous processes resulting in "clear" spaces between adjacent cells, and identification of a spectrum of changes from typical mesothelial cells to those showing reactive changes may also be possible. The golden rule in identifying the presence of atypical epithelial cells is to look for a "foreign population"; however, in some cases it may not be possible to accurately identify the cell type, and in these instances, immunohistochemical staining of cell block material and correlation with the findings of the surgical specimen may be of assistance in making the distinction between mesothelial and tumor cells [5].

Serous borderline tumors (SBT) of the ovary present with cohesive, smooth bordered papillaroid clusters of relatively small epithelial cells with scant cytoplasm, high nuclear/cytoplasmic ratios, and round to oval hyperchromatic nuclei with mostly inconspicuous nucleoli [5, 11] (Fig. 7.3a–d). Psammoma bodies are noted within the clusters in up to two thirds of cases [5] (Fig. 7.3e–g) but may also be seen in other benign conditions such as reactive mesothelial hyperplasia and endosalpingiosis. In one study, 41 of 112 cases of peritoneal fluids contained psammoma bodies associated with benign conditions [12].

Due to overlapping cytological features, the distinction between SBT and low-grade serous

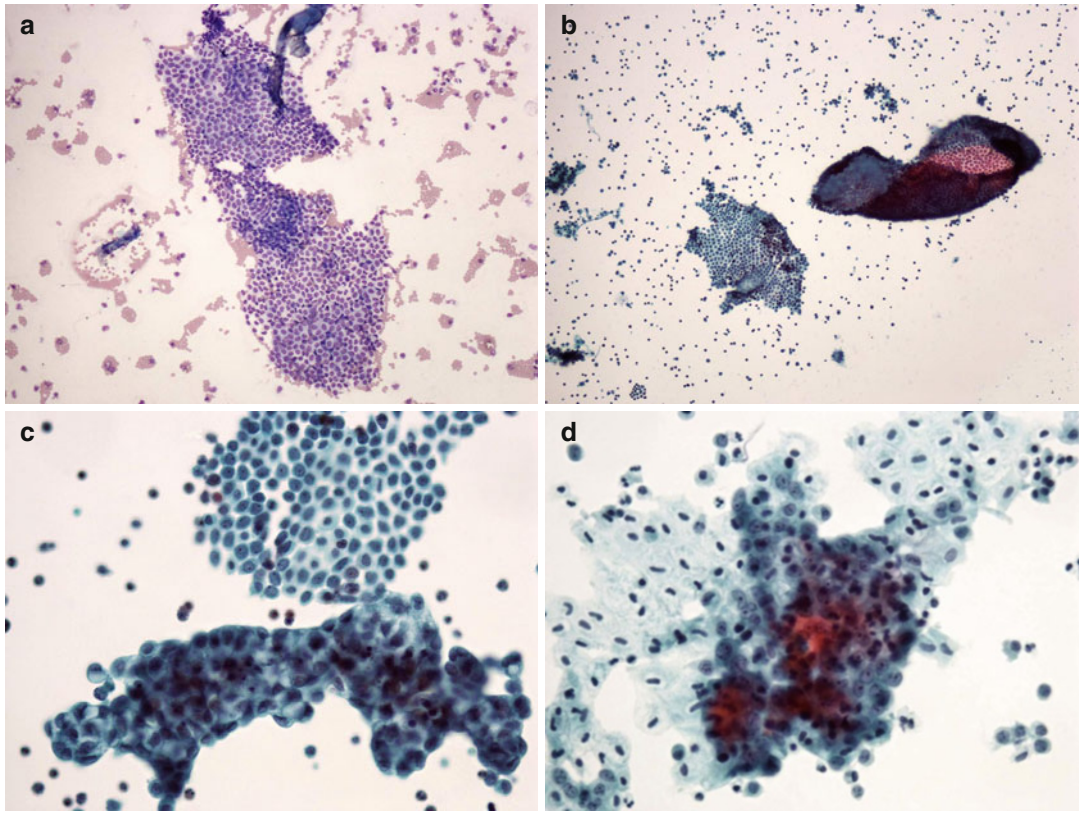


Fig. 7.1 (**a**) Flat sheet of evenly spaced bland resting mesothelial cells from a peritoneal washing sample (cytospin, MGG ×100). (**b**) Flat and folded (*right*) sheets of mesothelial cells amidst single mesothelial cells and inflammatory cells in a peritoneal washing sample (SurePath, Pap. ×100). (**c**) Flat sheet of nonproliferating mesothelial cells (*top*) adjacent to a 3-dimensional cluster of proliferating mesothelial cells, simulating two different cellular populations (SurePath, Pap. ×400). (**d**) Nonproliferating mesothelial cells with elongated nuclei (*top* and *left*) blending with a 3-dimensional group of proliferating mesothelial cells (ThinPrep, Pap. ×100)

carcinomas (LGSC) in cytology samples is not practically possible [13–16] as this distinction is made histologically by the presence of stromal invasion [17] which cannot be assessed in cytology samples. Therefore, these samples may be reported as showing features of an "atypical papillary serous epithelial tumor," or other similar terminology, and require correlation with the corresponding histology sample.

Benign Mimics

Endosalpingiosis, benign inclusions of tubal type epithelium that may be present in the pelvic peritoneum, can mimic SBT or LGSC and appear cytologically as relatively tight, cohesive simple tubular to papillary aggregates of cells, usually as a single layer with well-spaced nuclei, increased nuclear/cytoplasmic ratios, and indistinct nucleoli [18]. Cilia, when identified, are a helpful and perhaps essential diagnostic feature [5, 7] as, in their absence, overdiagnosis of a serous neoplasm may occur [9]. Although rare ciliated carcinomas, usually of ovarian origin, are described, for practical purposes, ciliated cells are benign until proven otherwise. Cilia may also be identified in cells derived from the Fallopian tube that may be present as small fragments and flat sheets of columnar epithelial cells. Psammoma bodies

may be associated with endosalpingiosis but should not, on their own, be used as a discriminating factor.

Histologically, *endometriosis* is characterized by the presence of endometrial stromal and glandular cells in association with hemosiderin-laden macrophages, although two out of the three components are sufficient for diagnosis. Cytologically, the stromal cells appear as loose aggregates of spindle cells, while the epithelial glandular cells form cohesive clusters, sheets, and tubular structures of bland, round to columnar cells with bland nuclei containing scant cytoplasm [19, 20] (Fig. 7.4a). However, the accurate categorization of cells as

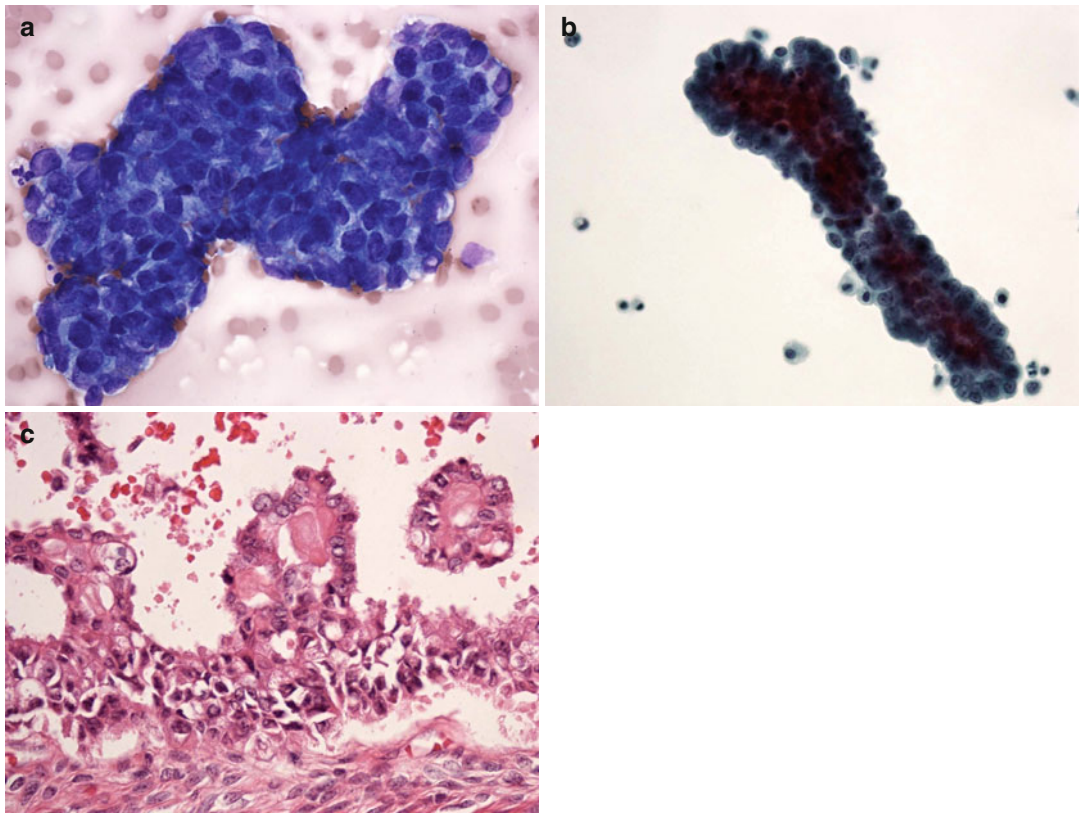
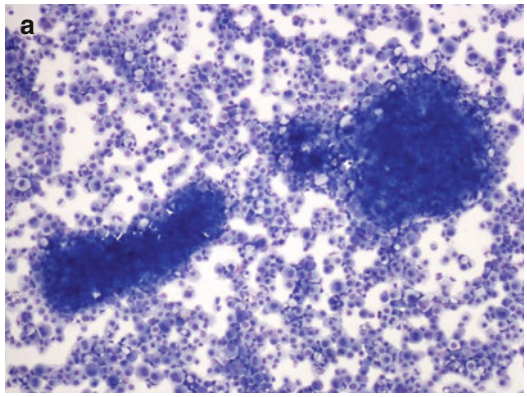


Fig. 7.2 (**a**) Cohesive cluster of reactive mesothelial cells with an irregular but smooth outline (cytospin, MGG ×600). (**b**) Tight papillaroid cluster of reactive mesothelial cells (ThinPrep, Pap. ×400). (**c**) Papillary mesothelial hyperplasia (same case as **b**) may be noted in histological sections on the surface of the omentum and ovary (illustrated) (H&E ×400)

Fig. 7.3 (**a**) Papillaroid clusters of cells from a serous borderline tumor (SBT) amidst single mesothelial cells (cytospin, MGG ×100). (**b**) Tightly cohesive papillaroid cluster of cells with predominantly high nuclear/cytoplasmic ratios and mildly atypical nuclei from an SBT (cytospin, MGG ×400). (**c**) Tight papillaroid cluster from an SBT with some cells showing vacuolated cytoplasm and lower nuclear/cytoplasmic ratios (ThinPrep, Pap. ×400). (**d**) Cluster of cells from an SBT composed of cells with scant cytoplasm, high nuclear/cytoplasmic ratios, and mildly atypical nuclei containing small nucleoli (cytospin, Pap. ×600). (**e**) Purple concentrically laminated psammoma bodies within vacuolated cells of an SBT (SurePath, Pap. ×600). (**f**) Several psammoma bodies associated with a papillaroid cluster of cells from an SBT (ThinPrep, Pap. ×400). (**g**) Clusters of translucent psammoma bodies in a cell cluster from an SBT (cytospin, MGG ×600)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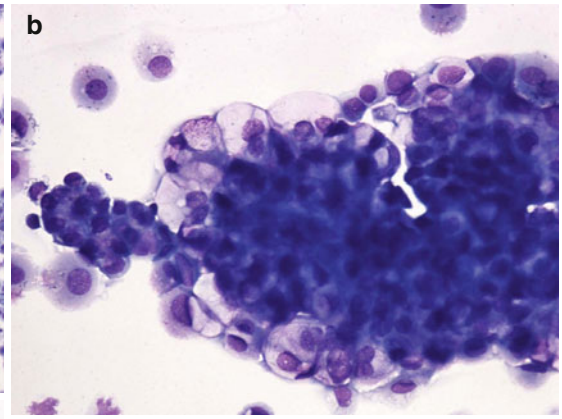
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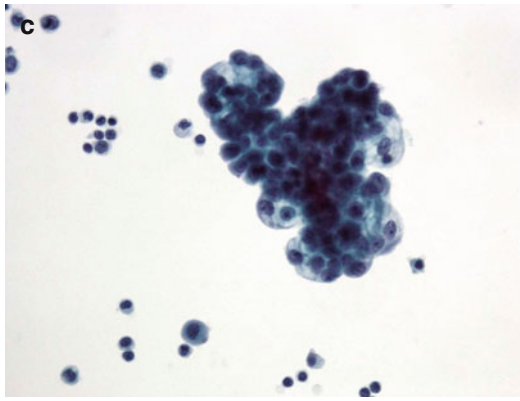
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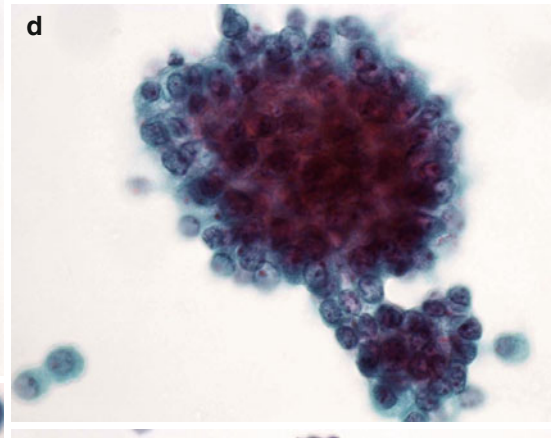
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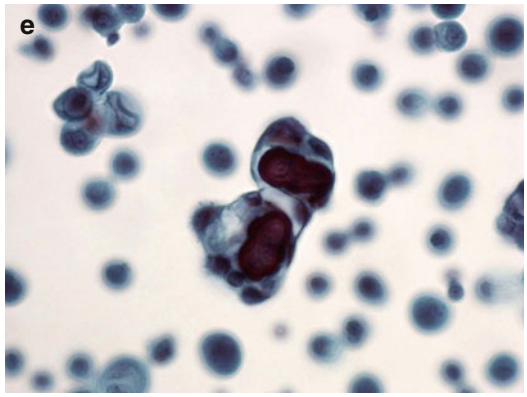
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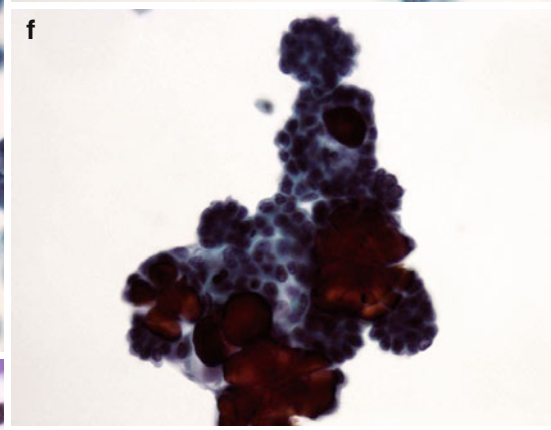
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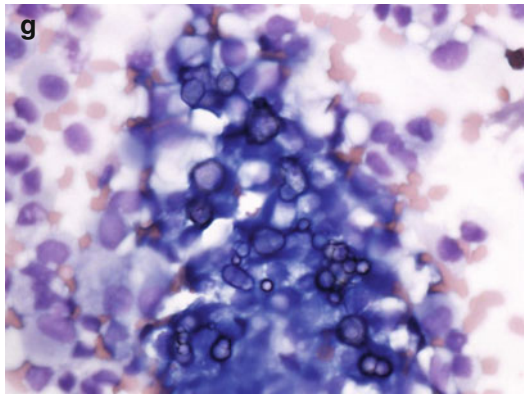
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endometrial may be difficult and a cell block may be of assistance in this regard [21]. Endometrial cells may also be present in fluid samples as a result of retrograde flow from the uterine cavity and, on their own, may not be due to endometriosis. However, the presence of hemosiderin-laden macrophages, with or without endometrial cells, is more likely to be associated with endometriosis [19]. The presence of endometriosis may also incite a mesothelial reaction, which may result in clusters of reactive mesothelial cells in association with hemosiderin-laden macrophages [5, 22].

The architecture of tissue fragments in proliferating mesothelial cells and mullerian inclusions has been noted to be less complex and more organized than that seen in SBT [7, 23]. Preparation of a cell block may assist in the distinction of tumors from benign mimics by enabling a better assessment of the architecture of cell groups and by allowing the use of immunohistochemistry to confirm the nature of the cells present [21] (Fig. 7.4b, c).

Collagen balls, consisting of collagen fragments overlain by mesothelial cells (Fig. 7.4d, e), are not infrequently seen in peritoneal washing samples and may originate from ovarian surface nodular stromal projections. They should not be mistaken for papillaroid fragments of an ovarian neoplasm or mucinous neoplastic cells [24].

High-Grade Serous Carcinoma

In contrast, high-grade serous carcinomas are usually readily recognizable, being characterized by 3-dimensional clusters with irregular outlines composed of larger cells with abundant vacuolated cytoplasm, pleomorphic nuclei with clumped chromatin, prominent nucleoli, and low nuclear/cytoplasmic ratios. The clusters are more dyshesive and single malignant cells are also present in the background [5, 11] (Fig. 7.5a–e). The presence of psammoma bodies is less common than in SBT [11].

Non-serous Primary Ovarian Carcinomas

The cytological subtyping of ovarian carcinomas in peritoneal washing samples is not generally required as this information is provided by the corresponding histology sample. However, certain features may provide clues to the subtype that may prove useful when these tumors are detected in ascitic fluid samples without a matching histology specimen.

Clear cell carcinoma presents with small clusters and single large malignant cells that have round nuclei with fine chromatin, prominent nucleoli, and abundant fine vacuolated cytoplasm (Fig. 7.6a–e). In addition, the cells characteristically surround basement membrane-like hyaline stromal material [25, 26]. Psammoma bodies have been reported in fluid specimens [26].

Endometrioid adenocarcinoma shows 3-dimensional clusters of malignant cells with pleomorphic nuclei containing coarse chromatin and prominent nucleoli. The cytoplasm may be relatively scanty or abundant and vacuolated (Fig. 7.7a–d).

Mucinous carcinoma may not have distinguishing features from other adenocarcinomas, depending on the degree of tumor differentiation and the presence or absence of mucin (Fig. 7.8a–d).

Immunohistochemistry as a Diagnostic Aid

In the context of peritoneal washing samples, frankly malignant cells are usually recognized by morphology alone. The role of immunohistochemistry in these samples is usually to distinguish between mesothelial cells and epithelial cells originating from SBT/LGSC or benign mimics, such as endosalpingiosis and endometriosis.

Various immunohistochemical markers have been used to identify mesothelial and epithelial cells. Mesothelial markers include calretinin, CK5/6, D2-40, WT-1, mesothelin, thrombomodulin, and HBME-1; however, none of these markers are entirely specific for mesothelial cells. Almost one third of serous carcinomas in one study showed varying degrees of calretinin positivity [27]. CK5/6 may be expressed in about half of ovarian serous carcinomas; however, reactivity in >50 % of tumor cells is limited to <15 % of

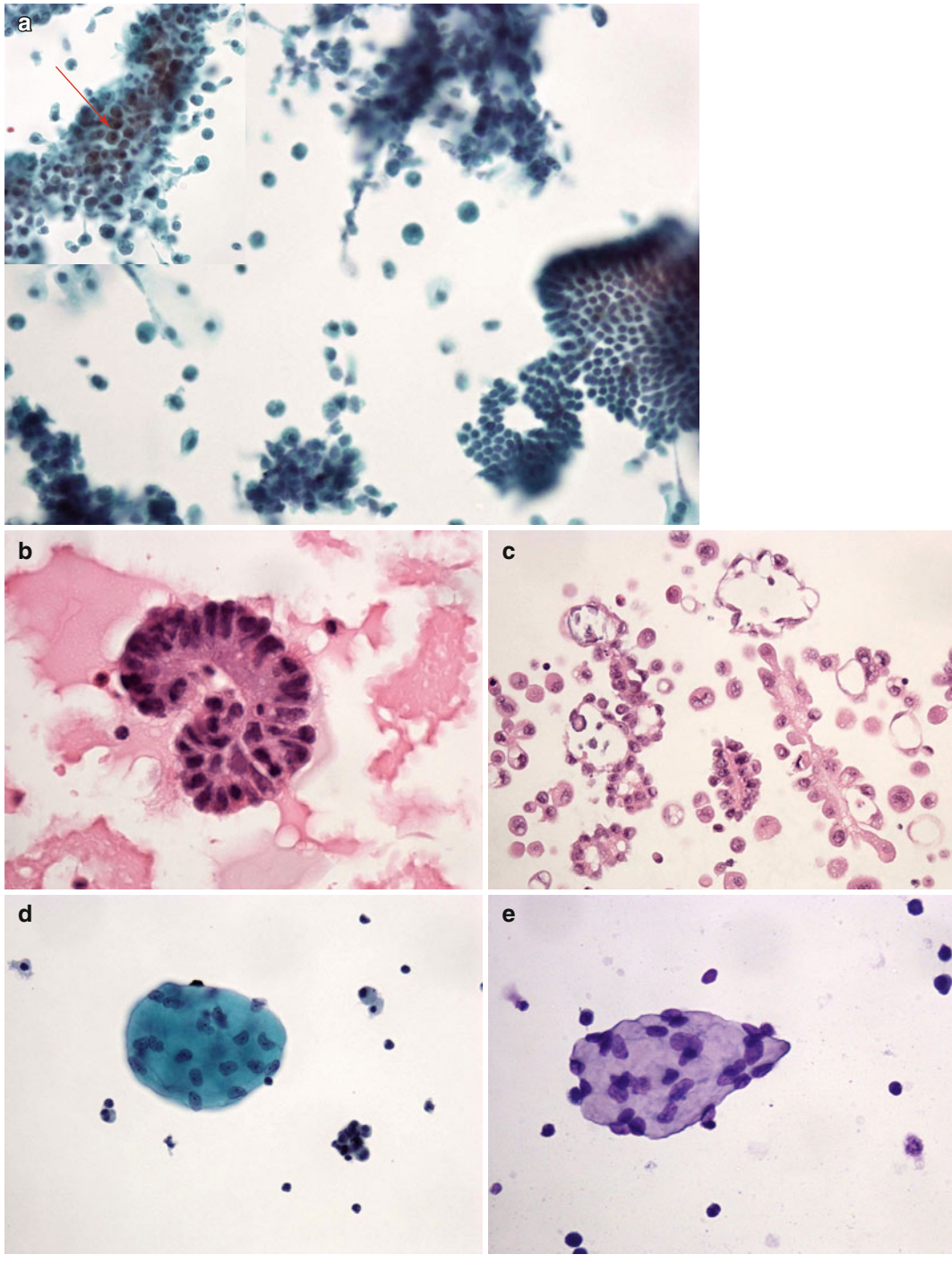


Fig. 7.4 (**a**) Endometriosis in a pelvic fluid sample showing a honeycomb sheet of epithelial cells with glandular border (*bottom right*), loose aggregates of spindled stromal cells (*centre top and bottom*) and haemosiderin-laden macrophages (*inset with arrow*) (SurePath, Pap. X400, inset X600). (**b**) Cluster of bland ciliated epithelial cells with a smooth outline in an agar cell block from endosalpingiosis (H&E ×600). (**c**) Papillaroid clusters of cells with high nuclear/cytoplasmic ratios and protuberant nuclei with fractured psammoma bodies within some of the clusters in an agar cell block from an SBT (H&E ×400). (**d**) Smooth contoured ball of collagenous tissue with overlying bland mesothelial cell nuclei (SurePath, Pap. ×400). (**e**) "Collagen ball" with a more irregular outline (cytospin, MGG ×400)

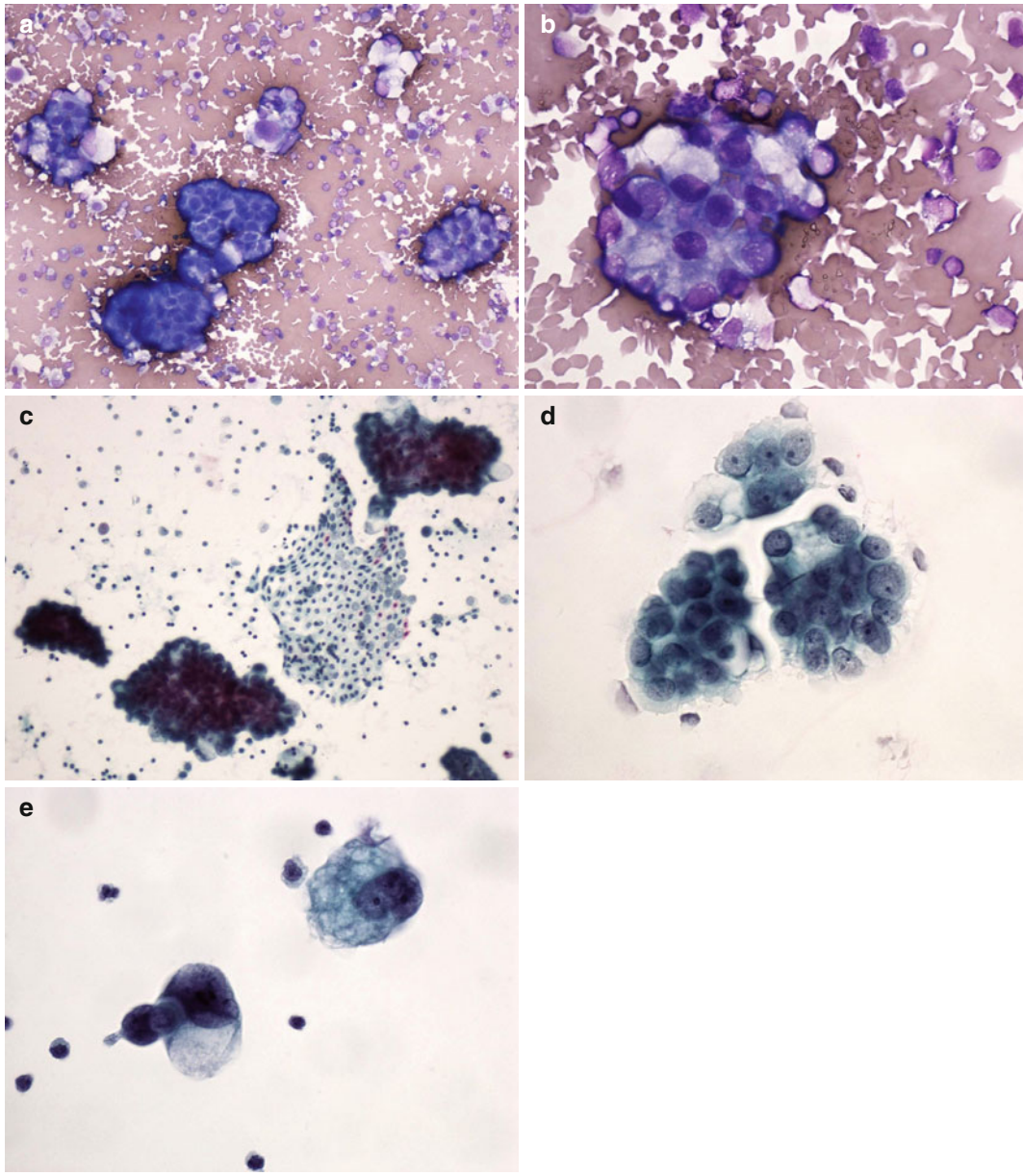


Fig. 7.5 (**a**) Large 3-dimensional clusters of malignant epithelial cells from a high-grade serous carcinoma (cytospin, MGG ×200). (**b**) Cluster of malignant epithelial cells with eccentric nuclei and abundant vacuolated cytoplasm (cytospin, MGG ×600). (**c**) Flat sheet of mesothelial cells (center) with adjacent 3-dimensional clusters of malignant epithelial cells (cytospin, Pap. ×200). (**d**) Clusters of malignant cells with high-grade nuclear atypia, prominent nucleoli, and low to high nuclear/cytoplasmic (N:C) ratios (cytospin, Pap. ×600). (**e**) Single malignant cells with eccentric, highly atypical nuclei, prominent nucleoli, abundant vacuolated cytoplasm, and low N:C ratios (SurePath, Pap. ×600)

cases [28]. It has been reported that D2-40 may stain more than half of tumor cells in 9–16 % of ovarian serous carcinomas, influencing its reliability as a marker to distinguish ovarian serous carcinoma from malignant mesothelial cells [28, 29]. In addition, WT-1 is expressed in >50 % of tumor cells in all ovarian serous carcinomas [28].

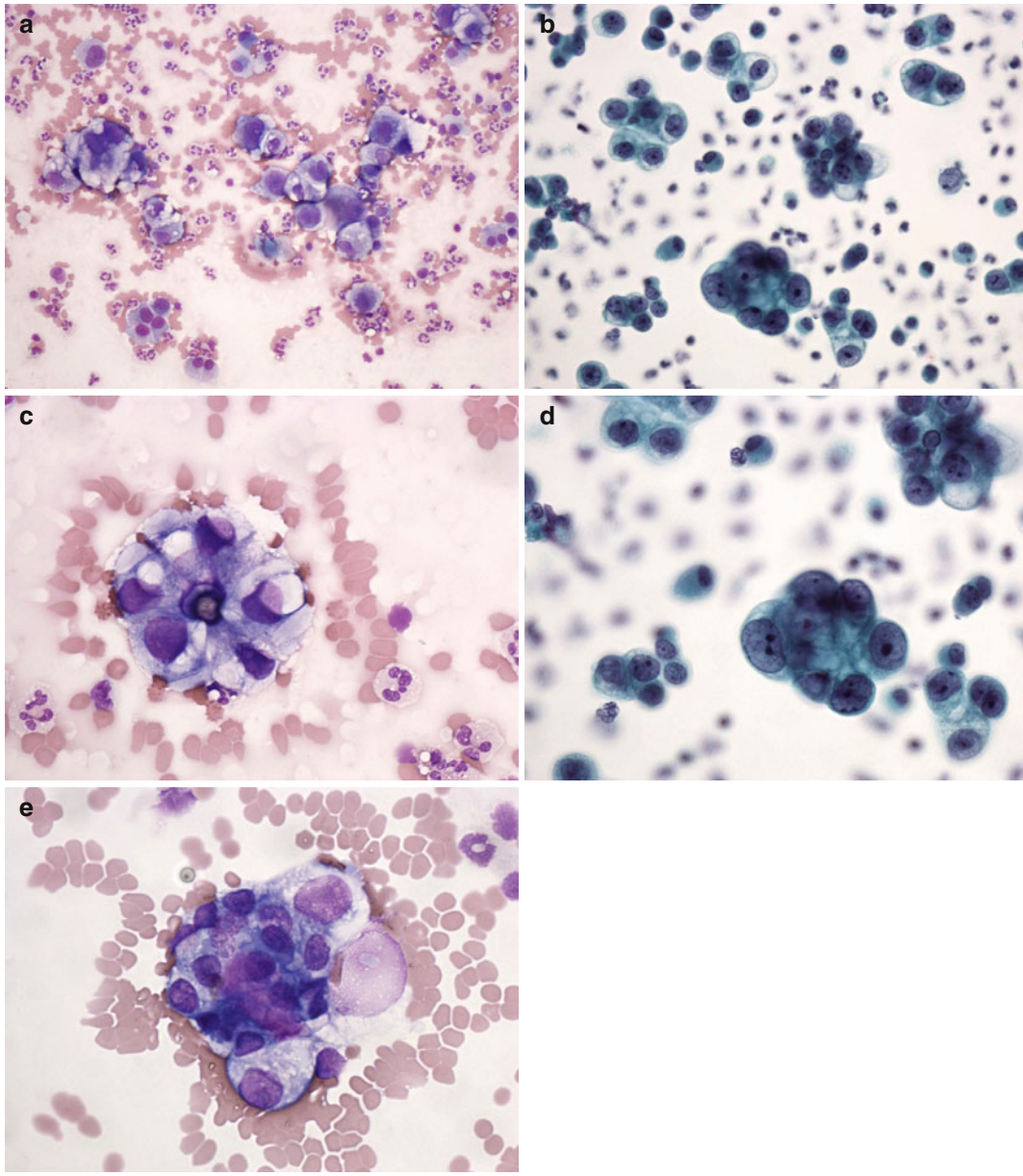


Fig. 7.6 (**a**) Single large malignant cells with vacuolated cytoplasm (cytospin, MGG ×200). (**b**) Small clusters of cells from an ovarian clear cell carcinoma with fine vacuolated cytoplasm, round nuclei, and prominent nucleoli (SurePath, Pap. ×400). (**c**) Cluster of clear cell carcinoma cells associated with a psammoma body, the latter not being specific only for serous tumors (cytospin, MGG ×600). (**d**) Small clusters and single clear cell carcinoma cells with prominent nucleoli, vacuolated cytoplasm, and ill-defined cell borders (SurePath, Pap. ×600). (**e**) Cluster of carcinoma cells with central hyaline stromal material (cytospin, MGG ×600)

Epithelial markers studied include MOC-31, BerEP4, B72.3, CEA, Leu-M1 (CD15), and BG8. MOC-31 and BerEP4 have been reported to be highly effective in the distinction between adenocarcinoma and mesothelial cells [30–33] with only focal staining reported in the latter [27].

For practical purposes, the use of a panel of four markers, namely, BerEP4, MOC-31, calretinin,

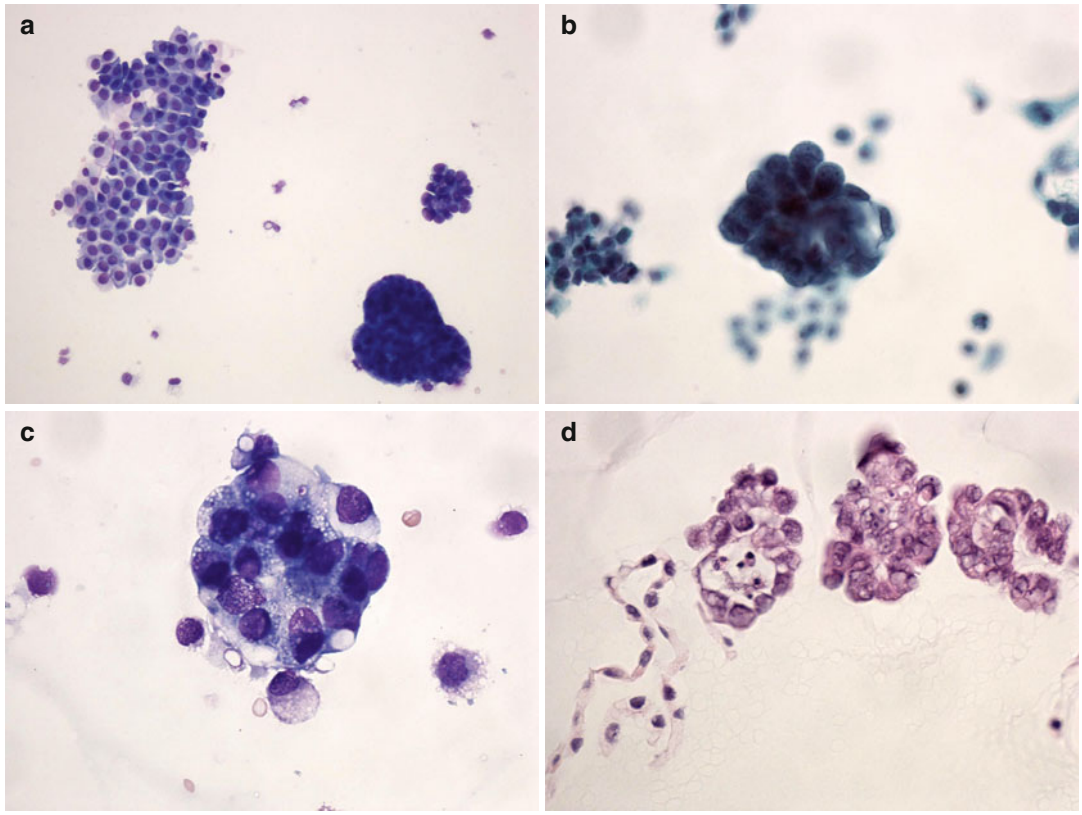


Fig. 7.7 (**a**) Flat sheet of mesothelial cells (*left*) in contrast to 3-dimensional clusters of cells from an endometrioid adenocarcinoma (*right*) (cytospin, MGG ×200). (**b**) Cell clusters from endometrioid adenocarcinoma may have relatively little cytoplasm with high nuclear/cytoplasmic ratios (SurePath, Pap. ×600). (**c**) Endometrioid adenocarcinoma may also shed cells with abundant vacuolated cytoplasm, low nuclear/cytoplasmic ratios, and eccentric nuclei (cytospin, MGG ×600). (**d**) Clusters of malignant endometrioid adenocarcinoma cells with relatively scant cytoplasm and prominent nucleoli adjacent to a strip of bland, nonproliferating mesothelial cells (*left*) in an agar cell block (H&E ×600)

and D2-40, usually enables the distinction between mesothelial cells and cells originating from serous tumors [27]. However, it should be remembered that BerEP4 and MOC-31 also stain most benign epithelial cells and *false-positive* diagnoses may arise in the context of endometriosis and endosalpingiosis if the morphological features of the cells are not carefully evaluated.

Diagnostic Accuracy

Various factors may influence the diagnostic accuracy of peritoneal washing cytology, including tumor characteristics, sampling technique, preparation techniques, and cytological interpretation [3]. It has been suggested that the ovarian tumor histological subtype influences the detection rate in pelvic washing cytology with serous carcinomas more likely to be detected than clear cell carcinoma [3]. However, as noted previously, the relative frequency of subtypes may be a confounding factor, with approximately two thirds of ovarian carcinomas being of serous type [6].

The presence of tumor cells in peritoneal washing samples of SBT correlates well with the presence of ovarian surface involvement [3, 9, 15]. In addition, cytology samples may be

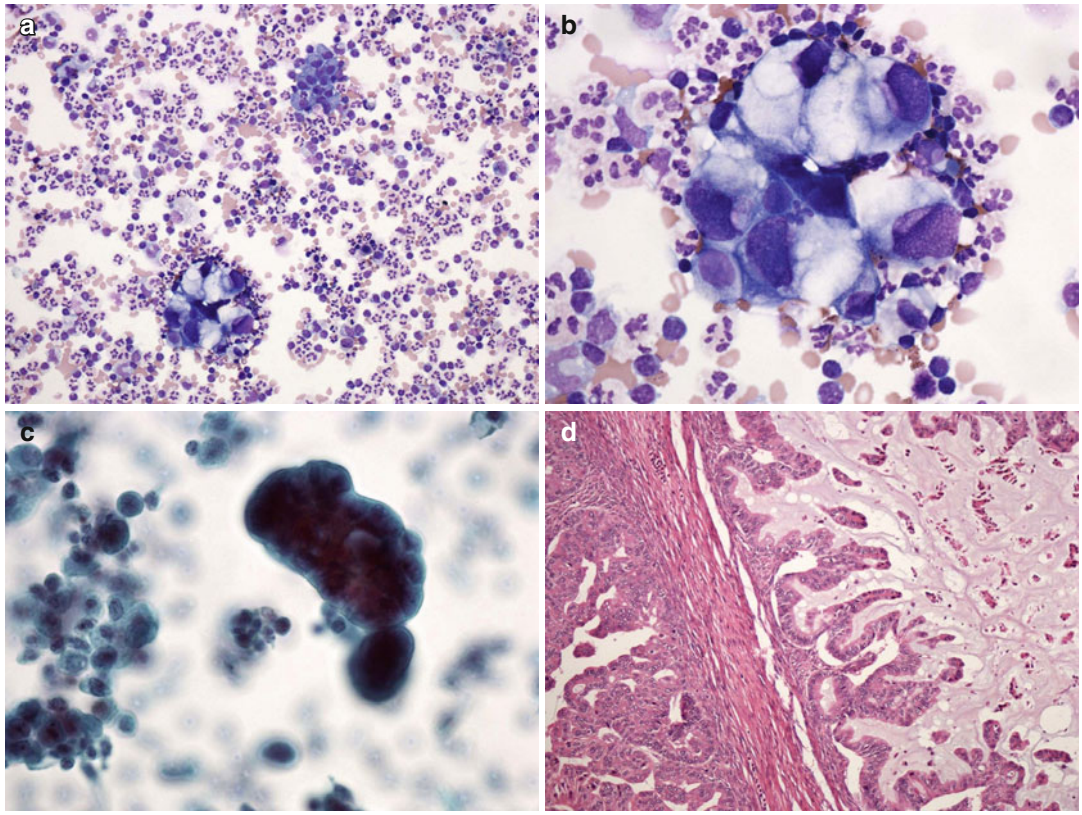


Fig. 7.8 (**a**) Sheet of benign mesothelial cells (*center top*) contrasted with a cluster of adenocarcinoma cells (*bottom*) amidst inflammatory cells (cytospin, MGG ×200). (**b**) High-power view showing a cluster of cells from a mucinous adenocarcinoma with eccentric nuclei and abundant vacuolated cytoplasm (cytospin, MGG ×600). (**c**) Cohesive cluster of hyperchromatic cells from a mucinous adenocarcinoma lacking intracytoplasmic mucin (SurePath, Pap. ×600). (**d**) Histology section of a mucinous ovarian carcinoma showing confluent glandular growth (*left*) and areas with luminal mucin and tufting of cell clusters (*right*) similar to those noted in the corresponding fluid specimen (**c**) (H&E ×100)

positive in the absence of histologically detectable ovarian surface or peritoneal involvement, making peritoneal washings a sensitive indicator of extra-ovarian disease [5, 9, 15]. Even in cases where there is macroscopic disease on the surface of the ovaries and a positive peritoneal washing will not alter the final tumor stage, it is still worthwhile performing peritoneal washings for cytology so that familiarity and expertise with the cytological appearances of these tumors is maintained.

As there are therapeutic and prognostic implications associated with upstaging based on a positive cytology result alone, it is important that a robust diagnostic process is in place in order to avoid false-positive results [34]. If there is diagnostic doubt based on the morphological appearances, immunohistochemistry should be performed to confirm the epithelial nature of the cells, bearing in mind the possible pitfalls due to benign mimics. Correlation with the histology findings of the surgical specimen [23] and discussion at a multidisciplinary meeting should be undertaken prior to deciding on the final stage of an individual case. In addition, as the cytological features of serous tumors arising in the ovary, Fallopian tube, and peritoneum are identical,

correlation with the radiological and histological findings is required to ascertain the most likely primary tumor site.

Role in Risk-Reducing Surgery

The use of peritoneal washing cytology in BRCA mutation carriers undergoing risk-reducing salpingo-oophorectomy has been advocated by some [35–37] but found to be of limited value and not recommended for routine practice by others [38]. A recent 10-year study of 117 patients found positive peritoneal washing cytology in less than 1 % of patients and highlighted the fact that, in view of the potential diagnostic pitfalls, critical review of a malignant result is required prior to therapy-related decision making. Although peritoneal washing cytology has the potential to detect occult malignancies, follow-up over longer periods from larger numbers of patients is required to ascertain the clinical significance of peritoneal cytology results in this setting [39].

Serous Fluids

The *diagnosis* of ovarian cancer is usually made on tissue samples submitted for histological analysis at the time of surgical treatment or biopsy material obtained prior to offering chemotherapy for advanced disease. In the latter group of patients, guidelines from the United Kingdom National Institute for Health and Clinical Excellence (NICE) advise that cytology may be utilized in cases where histology is not appropriate [40]. Serous fluid samples, generally ascitic fluid, may be obtained by percutaneous aspiration, with or without radiological guidance. In this clinical context, preparation of the sample may be tailored to ensure that sufficient material is available for preparation of a cell block to perform confirmatory immunohistochemistry. In addition, examination of serous fluid samples may be relevant in the *staging* of ovarian cancers, with malignant cells in fluid from the pleural cavities signifying the presence of stage IV disease. Finally, during follow-up of patients with treated ovarian cancer, malignant cells may be detected in serous fluid samples in the presence of *disease recurrence*.

The cytological features of ovarian cancer in serous fluid samples are well described in standard texts and have been outlined above. The malignant cells usually present as a "foreign population," distinct from the background mesothelial cells and inflammatory cells. Identification of the malignant cells is usually straightforward and the task is then to ascertain, by immunohistochemistry, the likely site of origin of the adenocarcinoma, as morphological features are often nonspecific. In some cases, malignant mesothelioma may also enter the differential diagnosis.

Metastatic Adenocarcinoma: Suggesting Possible Primary Sites

The approach to diagnosis of adenocarcinoma in ascitic fluid samples involves establishing the epithelial nature of the cells, using the markers outlined in section "Immunohistochemistry as a Diagnostic Aid" above, and then using panels of markers to target various primary sites. In female patients, the most likely tumors to present with malignant ascites are those arising from the female genital tract and peritoneum, upper and lower gastrointestinal tract, and pancreatobiliary system; however, breast carcinoma may also metastasize to the abdominal cavity.

Non-mucinous ovarian tumors show a CK7 +/ CK20 – profile and usually express CA125 and WT-1 to varying degrees. Clear cell carcinoma of the ovary, however, may not have a distinctive immunoprofile and is usually negative for CA125 and WT-1 [41]. Recently, PAX8 has been shown to be a useful addition as a good marker for tumors of Müllerian origin [42] with positivity in serous, endometrioid, and clear cell carcinomas [43]. High-grade serous carcinomas show proportionately more p53-positive cells than low-grade serous tumors [44]. It should be noted that the immunoprofile of ovarian serous tumors is identical to that of tumors originating in the Fallopian tube and peritoneum. As such,

distinction of the site of origin requires correlation with radiological findings, and the cytology report may be worded to the effect that the tumor is consistent with origin from any of these sites. Endometrioid ovarian carcinomas express CA125 and estrogen receptor (ER) but are negative for WT-1 and p53. Histologically, they may be mimicked by metastatic *colorectal carcinoma*. Clues to this diagnosis in ascitic fluid samples are malignant cells with a columnar morphology and background necrotic debris, with a characteristic immunoprofile (Fig. 7.9a–g). Lobular *breast carcinoma* spreads to the ovary more commonly than ductal carcinoma [45] and may present with bilateral ovarian masses. In fluid samples, these tumors often present as single cells or small chains and may contain intracytoplasmic lumina imparting a targetoid appearance (Fig. 7.10a, b).

Useful immunohistochemical panels that may be utilized in the workup of malignant ascitic fluid samples are outlined in Table 7.1. In addition to those markers outlined in the table, gross cystic disease fluid protein 15 (GCDFP-15) has been noted to stain almost half of metastatic breast carcinomas while being negative in almost all ovarian carcinomas [45]. This is helpful in conjunction with PAX8, which has not been reported to show positivity in breast carcinomas.

Mucinous Tumors

The relative rarity of primary ovarian mucinous carcinomas, as currently recognized, is subsequent to the appreciation that many mucinous ovarian tumors are metastatic from extra-ovarian sites [6]. Ovarian involvement by appendiceal mucinous neoplasms in the context of pseudomyxoma peritonei (PMP) may result in radiologically detected ovarian tumors with mucinous ascites. During macroscopic examination of ascitic fluid samples in these cases, a mucoid appearance may be noted, in which case, preparation of direct spread slides may be useful for demonstration of extracellular mucin. Clusters of atypical epithelial cells may be noted floating within the mucin, depending on the type of peritoneal involvement and immunohistochemistry assists in identifying the non-ovarian origin of the cells (Fig. 7.11a–f).

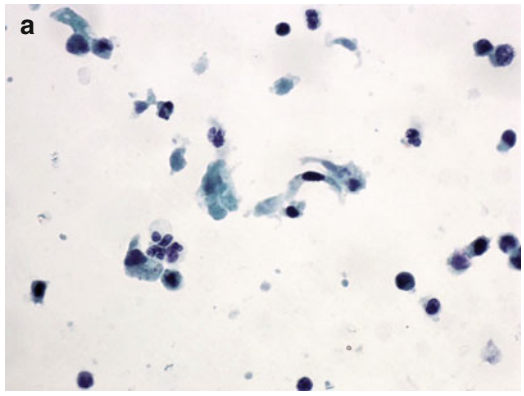
Mesothelioma

In some cases, it may be necessary to distinguish malignant mesothelioma (MM) from peritoneal carcinomatosis due to advanced ovarian serous or primary peritoneal carcinoma, as the clinical presentation is similar [46]. Ascitic or peritoneal fluid samples in MM are characterized by 3-dimensional clusters and single cells that retain the features of mesothelial cells, such as two-tone cytoplasm, intercellular windows, peripheral cytoplasmic blebs, and microvilli (Fig. 7.12a–c). The cells and clusters are usually larger than those seen in reactive mesothelial hyperplasia and the clusters have an irregular outline, in contrast to the smooth border seen in clusters of adenocarcinoma cells. However, morphological features are not specific and immunohistochemistry is useful in the distinction of MM from adenocarcinoma, as outlined in section "Immunohistochemistry as a Diagnostic Aid". In addition, PAX8 has been reported to be negative or show only focal/weak staining in peritoneal mesotheliomas, as opposed to the almost universal diffuse positivity noted in ovarian serous carcinomas, particularly those of high-grade type [47].

The application of immunohistochemistry in differentiating reactive and malignant mesothelial cells should be undertaken *subsequent* to demonstrating their mesothelial nature. Reactive mesothelial cells are positive for desmin and negative for epithelial membrane antigen (EMA). However, desmin is not a specific marker. Malignant mesotheliomas demonstrate strong membranous positivity with EMA and are negative for desmin. In addition, they may show strong diffuse nuclear positivity for p53 [48].

Non-epithelial Ovarian Tumors

The finding of cells from ovarian germ cell tumors and sex cord stromal tumors in serous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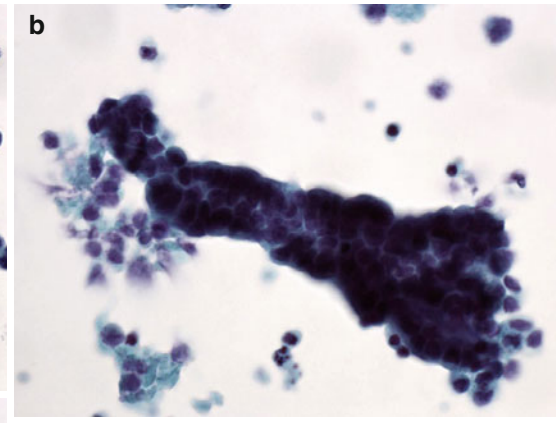
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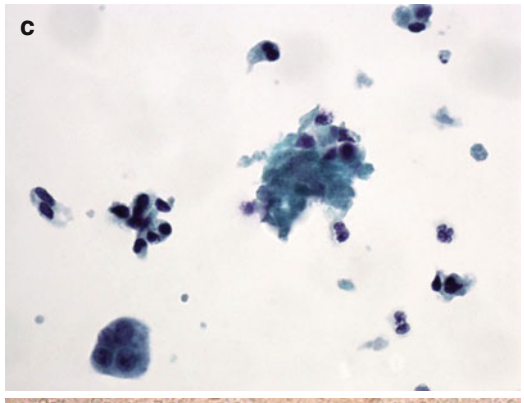
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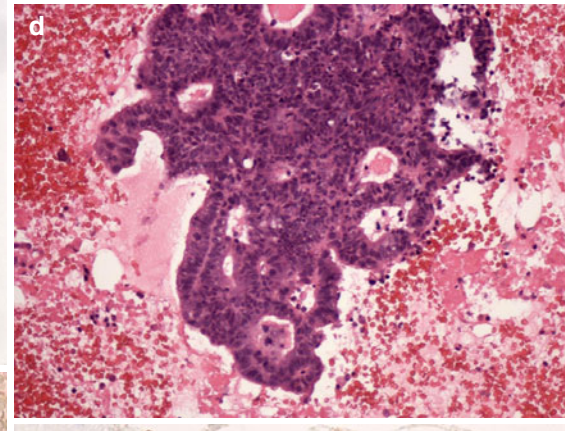
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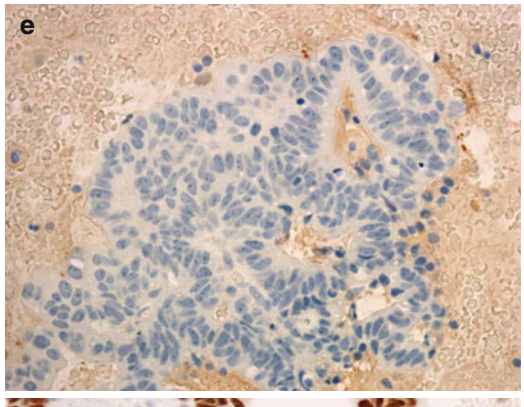
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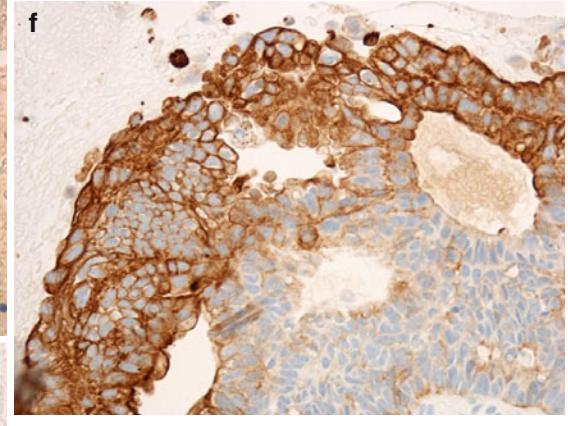
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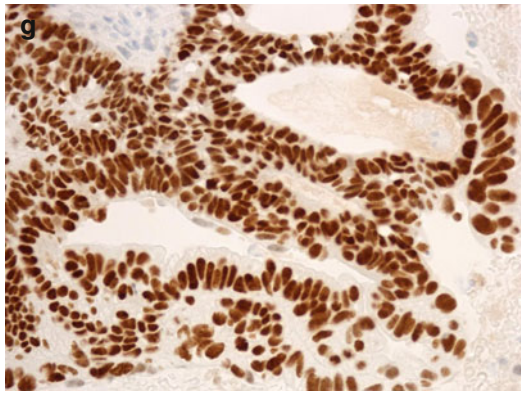
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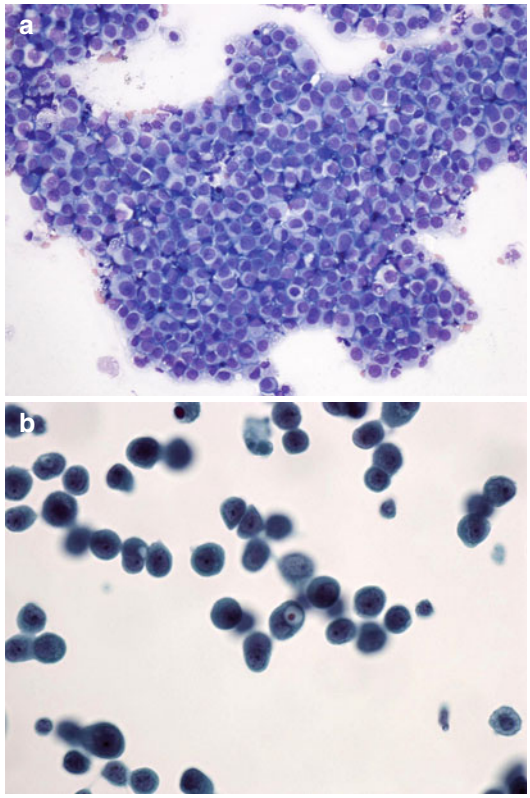


Fig. 7.10 (**a**) Dissociated population of single malignant cells from a metastatic lobular breast carcinoma (cytospin, MGG ×200). (**b**) Single, small dyshesive breast carcinoma cells, one of which (*center*) contains an intracytoplasmic vacuole containing a mucin droplet (SurePath, Pap. ×600)

fluids is uncommon and largely limited to case reports. The use of cell blocks on which immunohistochemistry can be performed is useful in these unusual cases [49]. Hair shafts and squamous cells, both of which may be surrounded by inflammatory cells, when found in peritoneal samples may be a clue to an *ovarian teratoma* (Fig. 7.13a–c) and have been observed even in the absence of clinically or microscopically detected tumor rupture [50]. *Dysgerminomas* may show cells in a sheetlike arrangement or as dyshesive single cells, with round nuclei containing macro- and multiple nucleoli amidst a background of lymphocytes (Fig. 7.14a–c) [51]. The cells may mimic adenocarcinoma or even malignant melanoma and lymphoma [49]; however, consideration of the age of the patient, as well as the judicious use of immunohistochemistry, should help resolve this differential diagnosis. Due to heterogenous microscopic appearances, *yolk sac tumors* may be difficult to diagnose in fluid samples and have been noted to closely resemble mucinous adenocarcinomas with 3-dimensional clusters of large malignant cells containing vacuolated cytoplasm [52]. Once again, the age of the patient is important. In addition, unlike adenocarcinoma, intracytoplasmic periodic acid-Schiff (PAS)-positive diastase-resistant globules may

Table 7.1 Immunohistochemical markers useful in determination of the primary site of metastatic adenocarcinoma presenting in ascitic fluid samples

Primary site	CK7	CK20	PAX8	CA125	ER	WT1	CDX2	CA19-9
Ovary (serous)	+	−	+	+	±	+	−	∓
Stomach	+	±	−	∓	−	−	∓	±
Colorectum	−	+	−	∓	−	−	+	±
Pancreatobiliary	+	±	−	∓	−	−	∓	±
Breast	+	−	−	∓	±	−	−	−

Fig. 7.9 (**a**) Single columnar malignant cells from a metastatic colorectal carcinoma (SurePath, Pap. ×600). (**b**) Papillaroid cluster of hyperchromatic, columnar malignant cells (SurePath, Pap. ×600). (**c**) Necrotic debris (*center*) with scattered columnar malignant cells with hyperchromatic nuclei (SurePath, Pap. ×600). (**d**) Clot section containing a tumor fragment showing acinar formation (H&E ×200). (**e**) Negative staining for CK7 in a metastatic colorectal carcinoma (×400). (**f**) Cytoplasmic positivity for CK20 (×400). (**g**) Diffuse nuclear positivity for CDX2 (×400)

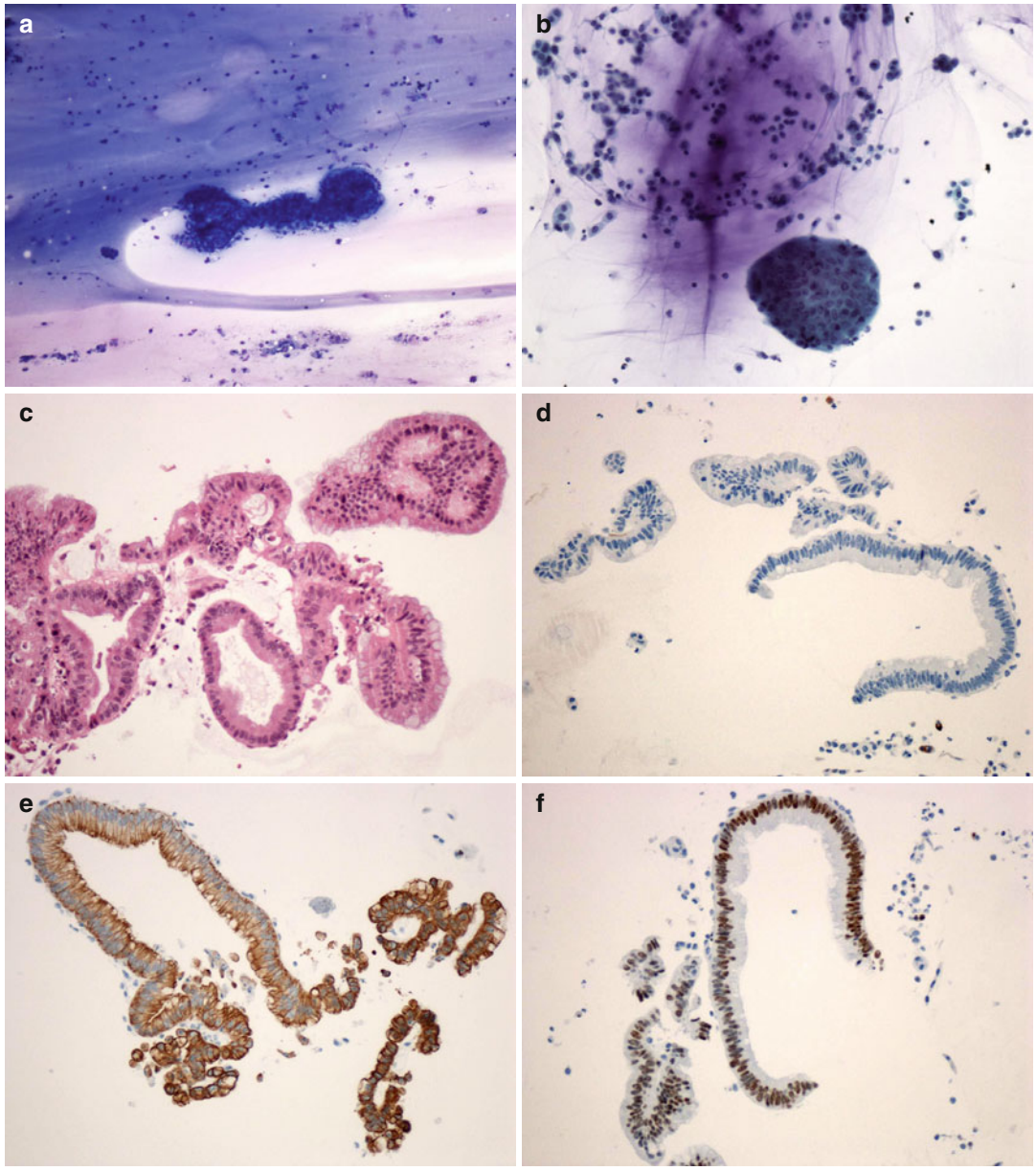


Fig. 7.11 (**a**) Cluster of epithelial cells amidst abundant extracellular mucin in a direct spread smear from a case of pseudomyxoma peritonei (MGG ×100). (**b**) Cohesive cluster of mildly atypical epithelial cells adjacent to extracellular mucin admixed with single mesothelial cells (SurePath, Pap. ×200). (**c**) Glands and papillaroid fragments of bland mucinous epithelium (agar cell block, H&E ×200). (**d**) Mucinous epithelium negative for CK7 (×200). (**e**) Diffuse cytoplasmic positivity in mucinous epithelium for CK20 (×200). (**f**) Diffuse nuclear positivity for CDX2 (×200)

be demonstrated [49], as well as cytoplasmic positivity for α-fetoprotein.

Sex cord stromal tumors are more rarely represented in fluid samples than germ cell tumors. *Adult granulosa cell tumors* (AGCT) are characterized by small clusters and single uniform, monotonous cells with nuclear grooves that may be overlooked in fluid samples or mistaken for mesothelial cells [53]. There may be fewer single cells than noted in

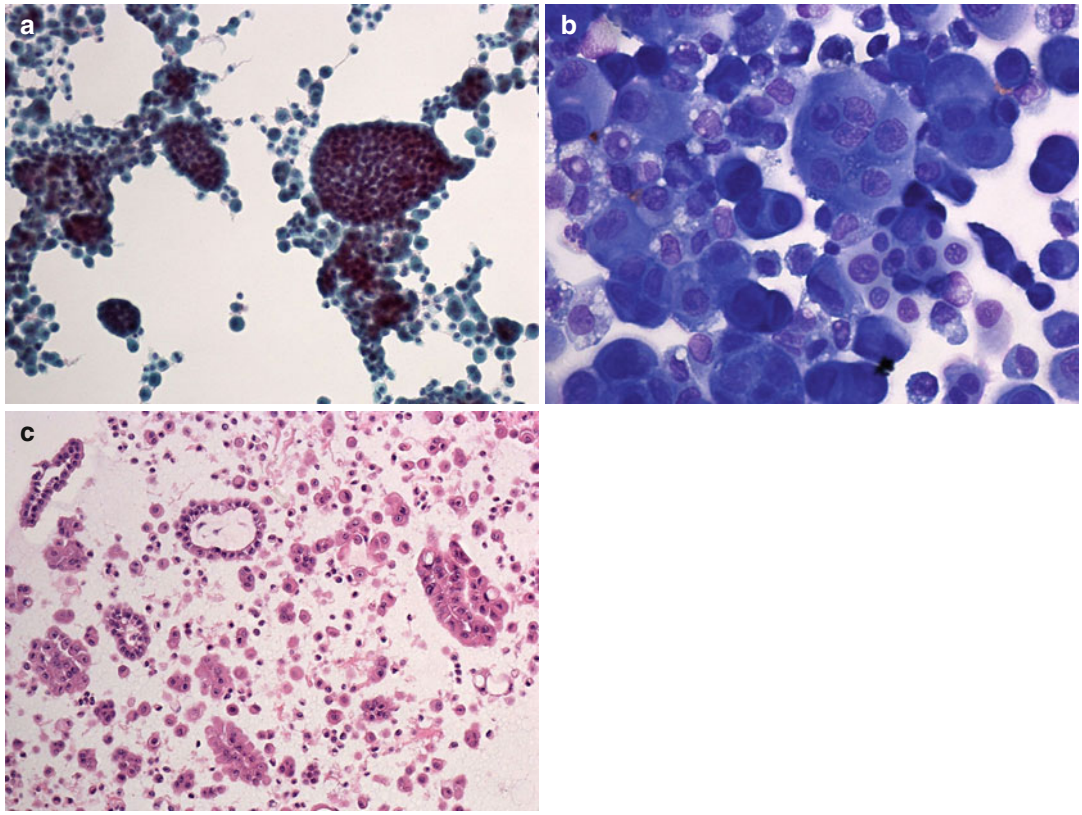


Fig. 7.12 (**a**) Large clusters and single malignant mesothelial cells (ThinPrep, Pap. ×200). (**b**) Clusters and single malignant mesothelial cells with peripheral cytoplasmic blebs (*center*) and intercellular windows (*top left*) (cytospin, MGG ×600). (**c**) Agar cell block containing papillary structures and single malignant mesothelial cells with prominent nucleoli (H&E ×200)

fine-needle aspiration samples [54], and Call-Exner bodies may not be identified in fluid samples. The latter, in combination with the finding that grooves may be more difficult to identify in fluid samples due to tighter cell clusters, may result in the diagnosis of AGCT not being considered [55]. Recurrent tumors show cells with more prominent nucleoli, vacuolated cytoplasm, and possibly mitoses and necrosis [54, 55]. *Juvenile granulosa cell tumors* are characterized by cells with more abundant eosinophilic cytoplasm and hyperchromatic, pleomorphic nuclei containing prominent nucleoli. These cellular features, in addition to the presence of mitotic figures, may simulate a carcinoma and represent a potential diagnostic pitfall [55].

Lymphoreticular Tumors

Ovarian involvement is a recognized manifestation of late-stage non-Hodgkin's lymphoma, at which time, malignant lymphoid cells may also be present in associated ascitic fluid samples (Fig. 7.15a, b).

Ovarian Fine-Needle Aspiration Cytology

The increased use of ultrasound and computerized tomography scans has resulted in ovarian cysts being detected with increasing frequency [56, 57]. This may be partly related to increasing numbers of young women undergoing investigation for

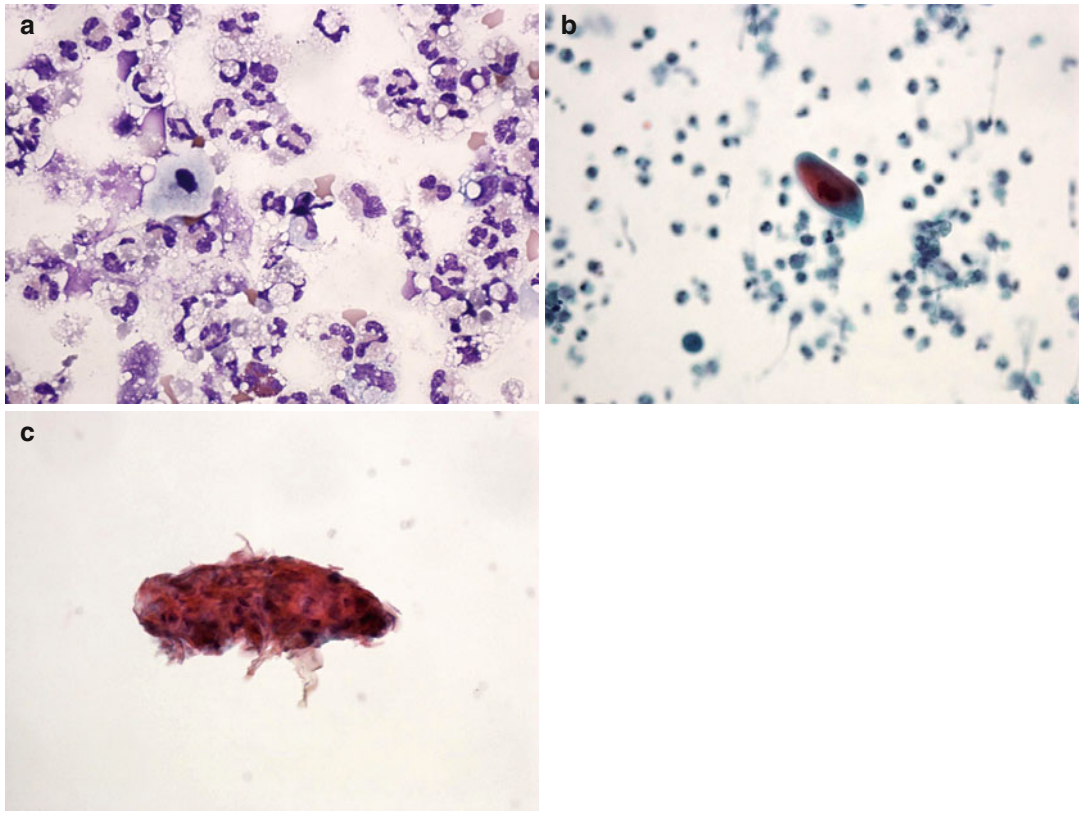


Fig. 7.13 (**a**) A single hyperchromatic squamous cell amidst neutrophils and macrophages (cytospin, MGG ×600). (**b**) A squamous epithelial cell amidst inflammatory cells in an ascitic fluid sample from a mature cystic teratoma case (SurePath, Pap. ×400). (**c**) An aggregate of nucleated and anucleate squamous cells in a peritoneal fluid sample (ThinPrep, Pap. ×400)

infertility [58, 59] and the widespread use of antenatal sonography [60], during which incidental adnexal cysts are diagnosed. Fine-needle aspiration (FNA) may be used to characterize ovarian lesions, particularly cysts, into nonneoplastic (functional and endometriotic cysts, inflammatory lesions) and neoplastic categories with the latter subdivided into benign and malignant entities. Ovarian FNA may be performed percutaneously under ultrasound guidance, transvaginally, transrectally, at the time of surgical evaluation of an ovarian cyst by laparoscopy or laparotomy and as 'bench' aspirates prior to surgical dissection, the latter serving as a means of increasing experience with cytological assessment prior to planned implementation of pre-operative FNA diagnosis [61]. Knowledge of the FNA route is important when reporting the cytology sample in order to be aware of the nature of potential contaminants that may be present within the specimen, consequent to the route of sampling.

FNA of the ovary is generally accepted as a harmless procedure that may be performed in an outpatient setting without the need for general anesthesia and that is associated with minimal complications [58, 62], although pelvic infection has been noted following transvaginal and transrectal approaches [63]. A potential risk of tumor spillage and seeding of malignancy secondary to an FNA procedure has been cited as a danger of such procedures; however, the literature relating to this is limited [64] and the magnitude of any possible risk is uncertain. However, caution dictates that for potentially malignant, radiologically

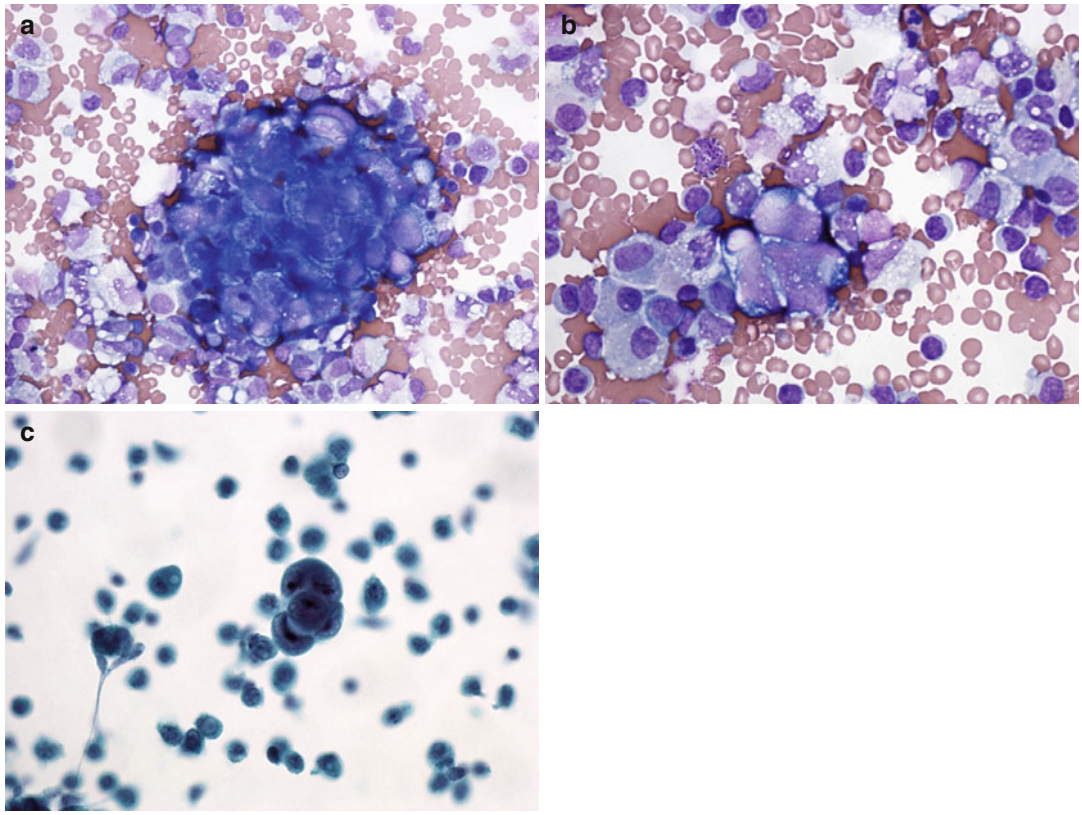


Fig. 7.14 (**a**) Cluster of cells from an ovarian dysgerminoma (cytospin, MGG ×400). (**b**) Three dysgerminoma cells with irregular nuclear outlines and scant cytoplasm amidst macrophages and small lymphocytes (cytospin, MGG ×600). (**c**) Small cluster of dysgerminoma cells with prominent nucleoli (SurePath, Pap. ×600)

early-stage ovarian tumors, FNA is generally not advocated.

In order to facilitate cytological assessment, good-quality preparations are required. Air-dried and alcohol-fixed slides are usually prepared from FNA samples and stained by May-Grünwald Giemsa (MGG) and Papanicolaou (Pap.) stains, respectively. These may be direct spread smears from solid lesions with the cyto-centrifuged sediment from cystic lesions used to prepare cytospins or smears. In addition, LBC techniques can be utilized and cell blocks prepared for ancillary techniques. The latter procedure is recommended as it may be the only way to establish the diagnosis [58]. In areas where infective ovarian pathology is prevalent, pus or necrotic material aspirated at the time of FNA may be sent for microbiological culture of mycobacterial and fungal organisms, with the diagnosis of an inflammatory lesion enabling nonsurgical management [65].

Utility of Ovarian FNA

There are various situations in which ovarian FNA may be of use. Women of reproductive age may particularly benefit from FNA *assessment of ovarian cysts*, which if functional in nature do not require surgical removal, thus preserving reproductive ability. In general, surgical excision is undertaken for all nonfunctional cysts [66]; however, surgical management has an associated cost to the patient and society and, in the context of benign ovarian disease, may represent overtreatment [57], particularly as more than one

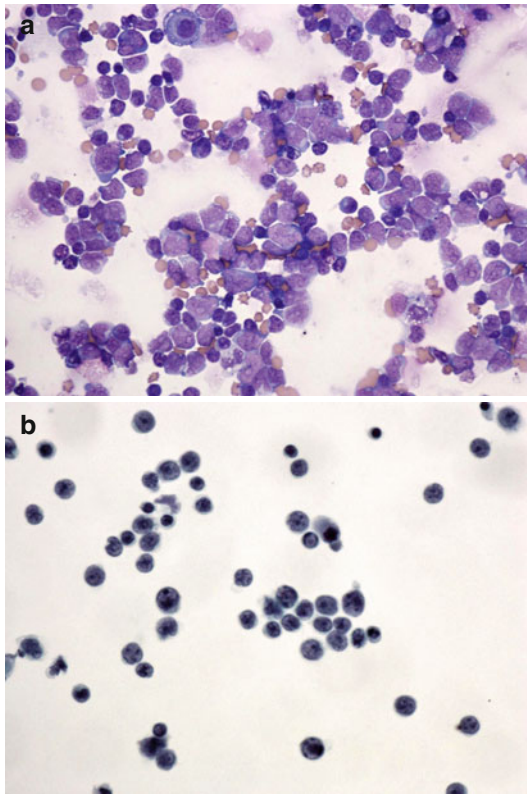


Fig. 7.15 (**a**) Large dyshesive malignant lymphoid cells with an occasional benign mesothelial cell (*top left*) in an ascitic fluid sample from a high-grade non-Hodgkin's lymphoma involving the ovary (cytospin, MGG ×400). (**b**) High-grade non-Hodgkin's lymphoma with cells containing large nuclei, prominent nucleoli, and scant cytoplasm (ThinPrep, Pap. ×600)

third of these cysts may show spontaneous resolution [67]. In patients with a low risk of malignancy index (RMI) score, it has been suggested that cytological assessment of fluid from simple ovarian cysts, particularly samples taken at the time of cyst removal, is of no clinical importance; however, this view may not be shared by treating clinicians [68]. In addition, fluid aspirated from ovarian cysts and sent for cytological examination at the time of surgical removal increases familiarity with and awareness of the cytological features associated with different types of ovarian cysts, which may prove useful in instances when a cytological diagnosis is required in a nonsurgical setting. Therapeutic transvaginal aspiration of probable benign ovarian cysts in high-risk surgical candidates is a practicable option [69], although a recurrence rate of 75 % was found in one series [57]. Pregnant patients may also be considered for management in this manner [70]. In addition, as for serous fluids, FNA may be useful in the follow-up of patients previously diagnosed with ovarian carcinoma who may have developed *recurrent or metastatic disease* [63] following treatment, possibly precluding second look surgery. Ovarian aspiration cytology also has a potential role to play in *screening* of BRCA-1 and BRCA-2 carriers at risk for the development of ovarian tumors, in combination with ultrasound and CA125 analysis [71].

Diagnostic Accuracy

The diagnostic accuracy of ovarian cyst FNA cytology is variable and may be affected by the sampling route, differences in specimen preparation, interpretative experience, as well as the clinical characteristics of the patient population studied [64, 72]. Due to the large range of ovarian tumor types, cytological assessment of FNA samples may represent a significant challenge [61, 73]. Specificity is reported to be high (67–100 %); however, sensitivity is more variable and often low (25–100 %) [56, 64], particularly in functional cysts and benign serous neoplasms [66], both of which may frequently result in nondiagnostic aspirates [74]. Specimen adequacy criteria are not well established, and different frequencies (18–80 %) of inadequate or nondiagnostic samples have been reported [56]. Some studies consider acellular samples or those with five or fewer cells to be inadequate [64] while others also assign samples containing only macrophages, red blood cells, and inflammatory cells [66] or cytologically uninterpretable aspirates due to degenerate diagnostic cells [58] to this category. Alternative terminology for samples yielding only sparse cells, including macrophages and degenerate cells, is a diagnosis of cyst contents not otherwise specified [75]. It should be noted that

malignancy cannot be excluded in an unsatisfactory or nondiagnostic sample [76].

Possible causes of false-negative results include low specimen cellularity, tumors which are largely cystic, and degenerative changes [65]. Lack of sampling of the epithelial cells lining a cyst is especially noted in serous cystadenomas, in which the lining may be atrophic [75, 77] and follicular cysts [66]. Multilocular cysts may result in nonrepresentative FNA sampling [74]. Careful cytological assessment is required to avoid a screening error in paucicellular samples [66]. False-positive diagnoses may arise in cellular aspirates and those showing cytological atypia [61], both of which may be noted in aspirates from follicular cysts [78–80].

Correlation with serum tumor markers [66] and ancillary techniques such as immunohistochemistry, flow cytometry, image cytometry, nuclear morphometry, and analysis of nucleolar organizer regions [81–83] may improve the diagnostic sensitivity and provide useful prognostic information in some ovarian lesions.

Ovarian Cysts

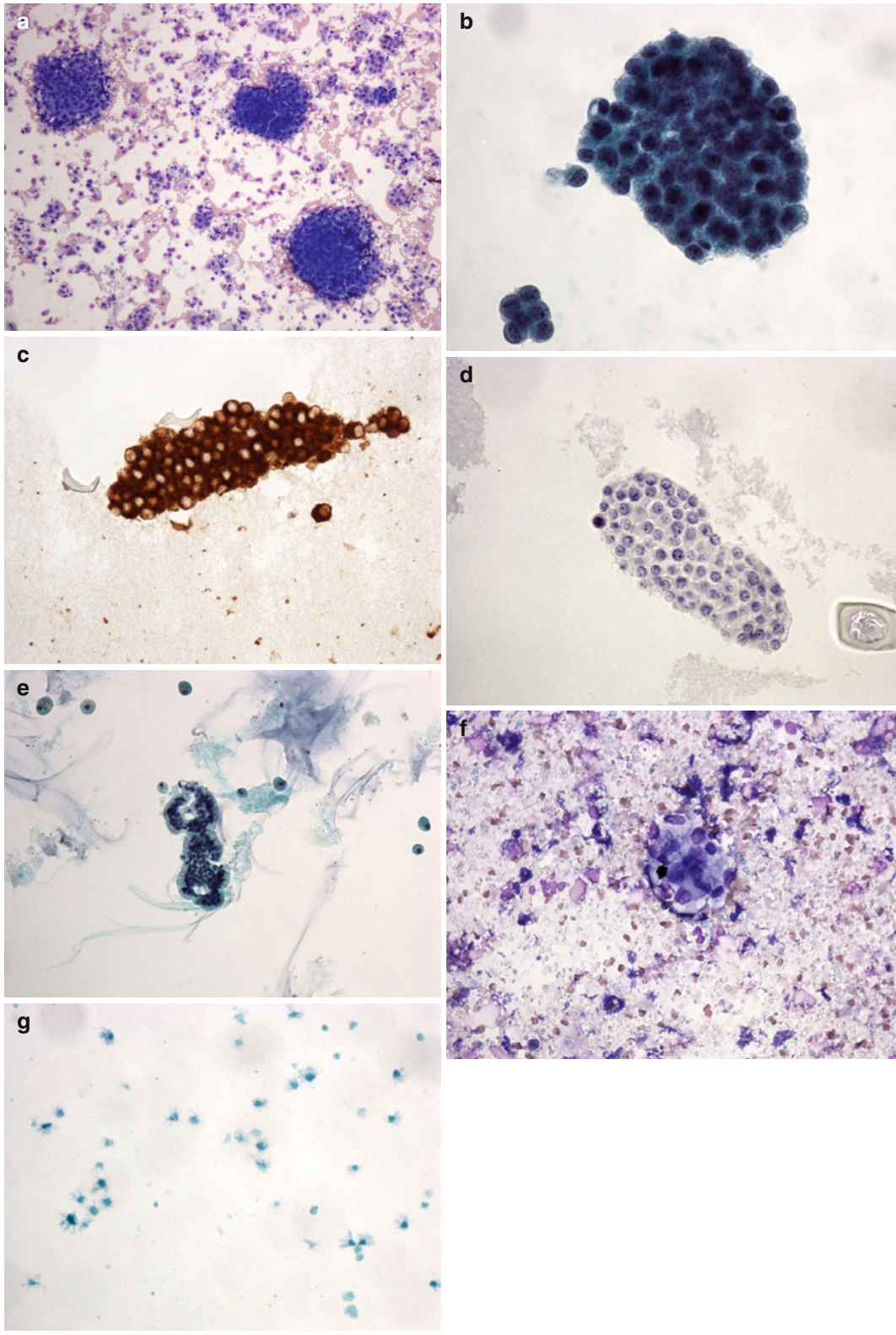
In the context of cystic ovarian lesions, the main cytological distinction to be made is between functional and nonfunctional epithelial-lined cysts. The former includes follicular and corpus luteum cysts, while the latter encompasses endometriotic cysts, cystic surface epithelial tumors of the ovary, and mature cystic teratomas. The macroscopic appearance of the cyst fluid can be variable and is not entirely specific to any particular type of cyst.

Functional cysts are characterized by granulosa cells which are small, have scant indistinct cytoplasm, and contain round to oval nuclei with coarsely granular chromatin imparting a "pepper-pot" appearance (Fig. 7.16a, b) [71, 74]. When degenerate, granulosa cells may mimic macrophages due to the presence of cytoplasmic microvesicles [74]. Luteinized granulosa cells are larger and have ill-defined cell borders with abundant foamy or granular eosinophilic cytoplasm and eccentric nuclei with fine chromatin and small nucleoli [71, 74]. In the context of corpus luteum cysts, luteinized cells may be seen in association with fibrin, degenerate erythrocytes [74], fresh blood, and hemosiderin-laden macrophages [84].

Follicular cysts pose a potential diagnostic pitfall due to high cellularity, cells with hyperchromatic nuclei containing conspicuous nucleoli, and the presence of mitotic figures, which may be numerous [63, 79]. A triple assessment including ultrasound, cytology, and cyst fluid estradiol-17β (E2) content allowed distinction of functional and nonfunctional cysts in 97.8 % of cases in one study, in which more than one third of patients were being investigated for infertility [58]. If all three parameters are negative, it has been suggested that expectant management is acceptable [77].

Immunohistochemical staining to detect inhibin excludes an epithelial cyst if positive but if negative does not exclude a functional cyst [85]. When used in conjunction with markers such as BerEP4 (Fig. 7.16c, d) and CK7 that would both be positive in epithelial cells and CD68, a macrophage marker [86], greater specificity is possible.

Endometriotic cysts yield brownish/chocolate-colored fluid [66] containing altered blood and hemosiderin-laden macrophages with endometrial stromal and/or epithelial cells present in some cases. The clinical details, macroscopic appearance of the cyst fluid, and cytomorphology may enable the diagnosis of such cysts to be suggested in many cases [65]. However, in the absence of a glandular epithelial component, the diagnosis may be difficult to establish [58], and caution should be exercised as corpus luteum cysts, torted follicular cysts, and various neoplastic ovarian tumors may also render bloodstained fluid containing pigmented macrophages [61]. A cytological diagnosis of endometriosis may enable commencement of medical therapy prior to surgical intervention and the aspiration procedure may, in addition, provide temporary relief from pain and compression symptoms [61]. Cellular degeneration may result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in atypia that can be misinterpreted, leading to a false-positive diagnosis [74].

Epithelial-lined cysts predominantly comprise those of serous and mucinous (Fig. 7.16e) type, which may range from benign to malignant in nature, with cellularity of aspirates increasing along the spectrum. Intracellular mucin may not always be appreciable in cells aspirated from mucinous tumors. Caution should be exercised in interpretation of transrectal FNA samples containing mucinous epithelium, which may represent contaminant rectal epithelium. The value of FNA cytology in the diagnosis of borderline ovarian neoplasms is limited [83]. Although the presence of cytological atypia may be evident, it is not possible to assess the presence or absence of stromal invasion in cytology samples, the latter being characteristic of borderline tumors [83]. As in serous fluid cytology, distinction of borderline and well-differentiated malignant tumors may not be possible [65, 87]. The variation in cytological features between benign and borderline/malignant serous tumors is greater than that seen between their more heterogenous mucinous counterparts [74]. It may not always be possible to ascertain the subtype of epithelial cells present (Fig. 7.16f); however, the main aim is to confirm the presence or absence of epithelial cells.

Fine-needle aspiration of *dermoid cysts* may yield thick yellow fluid containing squamous epithelial cells and keratinous debris. Foreign body-type multinucleated giant cells [66], sebaceous cells, or respiratory-type columnar cells may also be seen; however, hair shafts are rarely aspirated [58]. Transvaginal aspirates may contain contaminant squamous epithelial cells which on their own are not diagnostic of a dermoid cyst. Correlation with the clinical and radiological findings as well as careful assessment of the morphological features of all of the material on the cytology slide/s is required prior to suggesting a cytological diagnosis of mature cystic teratoma.

Paraovarian and paratubal cysts, such as hydatid of Morgagni and cystic mesonephric remnants, as well as hydro- and pyosalpinx may be aspirated as presumed ovarian cysts. These samples may contain sparse epithelial cells, usually of serous type [58, 66]. Correlation with radiological findings regarding the site of the cystic lesion is essential to avoid misdiagnosis as an ovarian serous cyst [66].

Detached ciliary tufts (Fig. 7.16g) are fragments of ciliated cytoplasm originating from epithelial cells of serous/tubal type that may be noted in aspirates from serous cystadenomas and cystadenofibromas, as well as paraovarian cysts and hydrosalpinges [88, 89]. The presence of these fragments is helpful in indicating the epithelial and thus nonfunctional nature of a cyst.

Solid Ovarian Tumors

The cytological features of the various ovarian tumors are well described in standard books of diagnostic cytopathology. In general, malignant *surface epithelial tumors* result in solid ovarian masses with a cytological diagnosis of malignancy being readily made, although accurate tumor subtyping may not always be possible. Recognition of metastatic carcinoma to the ovary is facilitated by clinical and radiological information, as well as the use of immunohistochemistry [65].

Accurate subtyping of *sex cord stromal tumors* may be difficult in FNA samples [65]. Diagnostically useful immunohistochemical markers for this group of tumors include

Fig. 7.16 (**a**) Three-dimensional clusters of follicular epithelial cells (cytospin, MGG ×200). (**b**) Tight cluster of hyperchromatic follicular cells with coarse "pepper-pot" chromatin and scant cytoplasm (ThinPrep, Pap. ×600). (**c**) Cluster of follicular cells showing strong positivity for inhibin (×400). (**d**) Follicular cell cluster negative for BerEP4 (×400). (**e**) Cluster of bland mucinous epithelial cells with honeycomb architecture amidst extracellular mucin and foamy macrophages in an aspirate from a mucinous cystadenoma (ThinPrep, Pap. ×400). (**f**) Cluster of epithelial cells from an ovarian cyst fluid aspirate that is difficult to morphologically subtype (cytospin, MGG ×400). (**g**) Detached ciliary tufts in an ovarian cyst fluid aspirate (SurePath, Pap. ×600)

calretinin, inhibin, steroidogenic factor 1, and WT-1 [90]. The diagnosis of adult granulosa cell tumor (AGCT), the most commonly aspirated tumor in this group, is often possible cytologically. It should be noted, however, that although nuclear grooves are characteristic of these tumors, they may also be noted in proliferating Brenner tumors and sex cord tumor with annular tubules (SCTAT). Call-Exner bodies, when present, may aid in the differential diagnosis, as well as the use of immunohistochemistry [91]. The diagnosis of *germ cell tumors* (GCT) may be made by FNA cytology with immunohistochemistry aiding in tumor subclassification and distinction from primary ovarian carcinomas. Dysgerminoma stains positively with OCT4 and placental alkaline phosphatase (PLAP), embryonal carcinoma with OCT4, human chorionic gonadotropin (hCG), and α-fetoprotein (AFP) and yolk sac tumor with AFP [41]. The proportion of different elements present in mixed germ cell tumors may affect sampling and therefore diagnostic accuracy [92].

Intraoperative Cytology

The use of cytology in the evaluation of ovarian neoplasms at the time of surgery may be complementary to frozen section assessment in cases where a definite preoperative diagnosis has not been possible. The diagnostic results may have surgical implications in young patients desiring preservation of fertility [93]. Cytological preparations may be obtained by needle aspiration, touch imprints, or surface scrapings from the surgical specimen and have the advantages of being relatively quick and easy to prepare with sampling of a wider tumor area and preserved cellular detail unaffected by freezing artifact. However, frozen section diagnosis is reported to be more accurate than cytology smear preparations in most cases, with the latter providing a supporting role [94–96] and not being in widespread use. Imprint cytology has also been performed on retroperitoneal lymph nodes, reportedly improving the staging of ovarian cancer [97].

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Overview of Epithelial Ovarian Carcinoma (EOC): Pathogenesis and General Considerations

8

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Abstract

Epithelial ovarian carcinomas (EOCs) comprise a heterogeneous group of neoplasms, the five most common subtypes being high-grade serous, low-grade serous, endometrioid, clear cell, and mucinous. In recent years, our understanding of the underlying pathogenesis and initiating molecular events in the different tumor subtypes has greatly increased, and although EOC is often considered clinically as one disease, there is now an increasing realization that the various subtypes have a different natural behavior and prognosis. Although at present, adjuvant therapy is mainly dependent upon tumor stage and grade rather than cell type, this is likely to change in the future with the development of new chemotherapeutic agents and targeted therapies against specific tumor subtypes or even specific molecular abnormalities. It is now firmly established that there are two distinct types of ovarian serous carcinoma, low grade and high grade, the former being much less common and arising in many cases from a serous borderline tumor. Low-grade and high-grade serous carcinomas represent two distinct tumor types with a different underlying pathogenesis rather than low-grade and high-grade variants of the same neoplasm. There is now emerging and compelling evidence that many high-grade serous carcinomas (by far the most common subtype of EOC) actually arise from the epithelium of the distal fallopian tube. Primary ovarian mucinous carcinomas are relatively uncommon, mostly unilateral and stage I, and largely of so-called intestinal or enteric type. Most arise in a stepwise manner from a preexisting mucinous cystadenoma and mucinous borderline tumor. Endometrioid and clear cell carcinomas typically present as low-stage neoplasms and in many, or most, cases arise from endometriosis; the former are usually well differentiated, and there is now evidence that the

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majority of neoplasms reported in the past as high-grade endometrioid carcinoma are of serous type. WT1 is useful in this regard since it is a relatively specific marker of a serous phenotype. It is recommended that different subtypes of EOC are graded using different systems rather than employing a universal grading system. Since most serous carcinomas are likely to arise from the fallopian tube and endometrioid and clear cell carcinomas mostly evolve from endometriosis which, via a process of retrograde menstruation, is ultimately of endometrial origin in most cases, it can be considered that true primary EOCs are rare, analogous to the situation in the testis.

Introduction

In most developed countries, ovarian carcinoma is the second commonest malignancy of the female genital tract, following endometrial carcinoma. In the United Kingdom, ovarian carcinoma is the fifth most prevalent carcinoma in women with approximately 6,800 cases diagnosed each year. There are various malignant ovarian germ cell and sex cord-stromal neoplasms and other rare miscellaneous malignancies, but these are uncommon compared to epithelial ovarian carcinoma (EOC), and this overview relates to the latter group of neoplasms.

Most cases of EOC occur in postmenopausal patients, but premenopausal women are occasionally affected, especially those with a hereditary predisposition to develop ovarian cancer, for example, women with BRCA1 or BRCA2 germ line mutation or Lynch syndrome (hereditary nonpolyposis colorectal cancer syndrome). In children in the first two decades of life, the most common EOC is mucinous carcinoma. The majority of cases of EOC are diagnosed at advanced stage (III or IV), and the overall prognosis is poor with a 5-year survival of approximately 43 %, but this ranges from almost 90 % in stage I to less than 20 % in stage IV. There are several different morphological subtypes of EOC, and, although clinically often considered as one disease, it is now apparent and increasingly recognized by pathologists and surgical and medical oncologists that the different morphological subtypes have a different pathogenesis, are associated with distinct molecular alterations, and have a different natural history and prognosis [1–6]. Many clinical and pathological studies lump the different morphological subtypes together with the result that it is difficult to tease out the behavior of the individual tumor subtypes; with our current state of knowledge, this is no longer appropriate. Given these factors and the realization that some tumor subtypes, for example, clear cell, mucinous, and low-grade serous, do not respond well to traditional ovarian chemotherapeutic agents and that ongoing trials, for example, the mucinous EOC (mEOC) trial [7], are investigating the efficacy of different agents in some of these tumor subtypes, it is clear that accurate pathological typing of EOCs is becoming more important and may be critical in the future in directing therapy. It is anticipated that targeted therapies will become available in the near future against individual tumor subtypes, sometimes based on the presence of specific molecular abnormalities. To this end, it is recommended that central pathology review becomes mandatory in ovarian carcinoma trials when treatment is dependent upon the morphological subtype or any other pathological parameter. Typing may also be of importance in directing investigations to confirm or exclude an inherited genetic condition; for example, a young woman (or indeed any woman) with a high-grade serous carcinoma could have an underlying BRCA1 or BRCA2 germ line mutation, while a clear cell or endometrioid carcinoma may be a manifestation of underlying Lynch syndrome. It is also possible that typing may become important in clinically stage I EOCs in determining the need for

lymphadenectomy since it has been shown that serous carcinomas are more likely than other subtypes to be associated with lymph node metastasis in clinically presumed stage I disease [8].

In this chapter, an overview of the major morphological subtypes of EOC is presented. Given that this chapter is an overview, there will be some overlap with other chapters which deal with the individual neoplastic subtypes. Problematic areas in tumor typing are also discussed. Although typing of most cases is straightforward on morphology alone, immunohistochemistry may assist in problematic cases and this is discussed where relevant.

Relative Frequencies of Major Morphological Subtypes of EOC

The major morphological subtypes of EOC are serous, endometrioid, clear cell, and mucinous [9]. Other subtypes include transitional and undifferentiated and mixed EOCs occasionally occur. Primary ovarian squamous carcinomas also occur; these are rare and usually arise in a dermoid cyst or more uncommonly in association with endometriosis or a Brenner tumor. Two relatively recent population-based studies which have included central pathology review using modern diagnostic criteria have provided updated information regarding the relative frequencies of the major subtypes of EOC [10, 11] (Table 8.1). It can be seen that serous is the most common followed by clear cell and endometrioid which occur with approximately equal frequency. Mucinous carcinomas are less common, accounting for approximately 3 % of EOCs. This represents a change from most prior studies where mucinous carcinoma was the second commonest subtype and accounted for approximately 12 % of EOCs [12], a much higher frequency than in the two recent studies; reasons for this decline in the frequency of mucinous carcinoma are discussed later in this chapter. In some institutions, mucinous carcinomas account for more than 3 % of EOC, and this is likely a reflection of the fact that there is significant interobserver variability among pathologists in the distinction between a mucinous borderline tumor of intestinal type at the upper end of the spectrum and a well-differentiated mucinous adenocarcinoma of intestinal type. If the pathologists in a particular institution routinely diagnose these problematic neoplasms as adenocarcinoma, then the prevalence of primary mucinous adenocarcinomas is likely to be greater than 3 %. The two population-based studies referred to also indicate an increase and decrease in the frequency of serous and endometrioid carcinoma respectively; this is likely a reflection of the fact that the distinction between a high-grade serous and high-grade endometrioid carcinoma was in the past poorly reproducible [13–17], and there is now a realization that many neoplasms which were previously diagnosed as advanced-stage, high-grade endometrioid carcinoma were, in fact, of serous type (discussed later). When divided into early stage (stage I–II) and late stage (stage III–IV), it can be seen that serous, clear cell, and endometrioid carcinomas are approximately equally represented in early stage, nearly all mucinous carcinomas are early stage, and a very high percentage of advanced-stage neoplasms are serous in type (Table 8.2). To put it another way, a high percentage of clear cell, endometrioid, and mucinous carcinomas are diagnosed at early stage, and in fact these tumor types (especially

Table 8.1 Relative frequencies of major subtypes of epithelial ovarian carcinoma (EOC) based on two recent population-based studies

68–71 % serous
3 % mucinous
9–11 % endometrioid
12–13 % clear cell
1 % transitional
6 % mixed

Table 8.2 Early-stage (I/II) versus late-stage (III/IV) distribution of subtypes of epithelial ovarian carcinoma based on two recent population-based studies

	Stage I/II (%)	Stage III/IV (%)
Serous	36	88
Clear cell	26	5
Endometrioid	27	3
Mucinous	8	1

endometrioid and mucinous) are usually confined to the ovary at diagnosis (stage I). It is also discussed later in this chapter that there are two distinct types of ovarian serous carcinoma, termed low grade and high grade, each with a distinct and different pathogenesis, and that high-grade serous carcinoma is much more common than low grade.

Morphological Alterations in EOC Secondary to Chemotherapy

For many years, the traditional management of advanced-stage EOC has been surgical debulking followed by adjuvant chemotherapy. However, in an increasing number of patients, upfront chemotherapy is administered, especially in those who are a poor operative risk or patients with miliary disease, extensive upper abdominal disease, or widespread metastasis (usually hepatic or pulmonary) where optimal debulking is not considered feasible. It is increasingly recognized that if total debulking is not considered feasible, upfront chemotherapy should be administered as the first line of treatment. In such cases, chemotherapy may or may not be followed by surgery. The morphological features of ovarian carcinomas treated by chemotherapy sometimes differ markedly from native tumor [18, 19]. Post-chemotherapy, many ovarian carcinomas (the vast majority will represent high-grade serous carcinomas since almost all advanced-stage ovarian malignancies are of this morphological subtype) have abundant clear or eosinophilic cytoplasm, and the nuclear features may be bizarre with multinucleate tumor giant cells [18, 19] (Fig. 8.1). There may be no residual tumor, or only small nests of residual neoplastic cells may be present because of pronounced chemotherapy effect with marked fibrosis, necrosis, inflammation, cholesterol cleft formation, hemosiderin deposition, and dystrophic calcification. Unless there is no or minimal response, it can be very difficult to type an ovarian carcinoma following chemotherapy and there is a tendency to misdiagnose some as clear cell carcinoma due to the abundant clear cytoplasm [18, 19]. However, helpfully markers which are characteristically expressed in serous carcinoma, such as WT1, are still positive. If upfront chemotherapy is being administered, a pre-chemotherapy tissue biopsy (usually a percutaneous radiologically guided biopsy or occasionally a biopsy obtained at laparoscopy) should be obtained for definitive typing, apart from in exceptional circumstances, rather than relying on cytology of the ascitic fluid in combination with serum CA125 levels and imaging. The procurement of a tissue biopsy, as well as facilitating tumor typing and excluding a metastasis from other sites, means that material is available for future studies and research and this may be important in management; for example, targeted therapies may be developed against specific proteins or molecular events, and if tissue is available, this will be useful in assessing whether the target protein or molecular abnormality is present within the tumor. Tissue obtained at

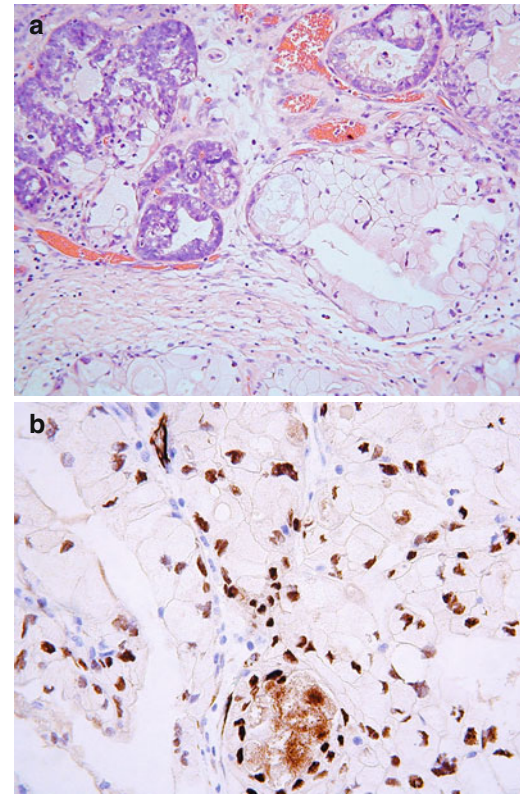

Fig. 8.1 High-grade ovarian serous carcinoma with focal clear cytoplasm post-chemotherapy; the morphological features may mimic a clear cell carcinoma, (**a**) but WT1 is diffusely positive (**b**)

different stages in treatment may also be useful in assessing tumor progression and response to therapy. The National Institute for Health and Clinical Excellence (NICE) guidelines in the United Kingdom have recommended a pre-chemotherapy tissue biopsy as the standard of care in patients with EOC who are undergoing chemotherapy as the first line of treatment [20].

Grading of EOC

Several grading systems are used for EOC, but grading is often poorly performed by pathologists, and in many studies the grading system used has not been specified [21–23]. Most of the grading systems are universal in that they can be applied to all the major morphological subtypes of EOC [21–23]; for example, the Shimizu-Silverberg system is based on the Nottingham grading system for breast carcinoma and uses three parameters, namely, the degree of nuclear atypia, the mitotic count, and the architectural features, specifically whether the predominant pattern of growth is glandular, papillary, or solid [21–23]. Each parameter is given a score of 1–3, and a grade is derived based on the summation of the scores (Table 8.3). However, although many pathologists use this or other universal grading systems, such as FIGO or World Health Organization (WHO), there is an increasing tendency to employ different grading systems for different morphological subtypes of EOC; this practice has been recommended in the Royal College of Pathologists Ovarian Cancer Dataset in the United Kingdom (Table 8.4) [24]. It is recommended that serous carcinomas are classified as low grade or high grade (see below), recognizing that these represent two distinct tumor types rather than low-grade and high-grade variants of the same neoplasm. Clear cell carcinomas are automatically regarded as grade 3, and endometrioid adenocarcinomas are graded according to the FIGO grading system used for endometrioid adenocarcinomas of the uterine corpus. There is no validated grading system for mucinous adenocarcinomas, but it is recommended that these are graded in an identical manner to endometrioid adenocarcinomas [24].

Table 8.3 Shimizu-Silverberg grading system for epithelial ovarian carcinoma (EOC)

Score	Predominant architectural pattern	Cytologic atypia	MFs/10 HPFs
1	Glandular	Slight	0–9
2	Papillary	Moderate	10–24
3	Solid	Marked	>/–25

Total score *3–5* grade 1, *6 or 7* grade 2, *8 or 9* grade 3

Table 8.4 Recommended grading systems for different morphological subtypes of epithelial ovarian carcinoma (EOC) (Royal College of Pathologist's Dataset)

Morphological subtype	Grading system
Serous	Low grade High grade
Endometrioid	FIGO grading system for uterine corpus adenocarcinoma
Clear cell	Automatically grade 3
Mucinous	FIGO grading system for uterine corpus adenocarcinoma

Morphological Subtypes of EOC

In the next sections, the major morphological subtypes of EOC are covered. The morphological and immunohistochemical features are not discussed in detail since these are presented in the relevant chapters.

Serous Carcinoma

The perceived relationship between benign, borderline, and malignant ovarian serous neoplasms was controversial and a source of confusion for many years. It has always been tempting to speculate that a continuum of ovarian serous neoplasia exists from benign to borderline to malignant. However, pathological evidence for this is lacking, and until relatively recently it was generally assumed that there was no firm relationship between borderline and malignant serous neoplasms, although occasionally the two were found to coexist. Recent studies clarified this issue and have convincingly demonstrated that there are two distinct types of ovarian serous carcinoma, low grade and high grade [1–6, 25–31]. Although termed low-grade and high-grade serous carcinoma, it is important to

emphasize that these are not two grades of the same neoplasm but rather two distinct tumor types with different underlying pathogenesis, molecular events, behavior, and prognosis. High-grade serous carcinoma is much more common than low grade by a factor of at least 10 to 1. Low-grade serous carcinoma is thought to arise in many cases in a stepwise fashion from a benign serous cystadenoma through a serous borderline tumor to an invasive low-grade serous carcinoma. Thus, there is a well-defined adenoma-carcinoma sequence. It has been suggested that a micropapillary architecture in a serous borderline tumor is an intermediate step between a usual serous borderline tumor and an invasive low-grade serous carcinoma [1, 31–33]. However, micropapillary architecture is not present in many serous borderline tumors with coexistent low-grade serous carcinoma; it is relatively uncommon to see areas of invasive low-grade serous carcinoma within a serous borderline tumor, and conversely in some low-grade serous carcinomas, a borderline component is not seen. Therefore it is not proven that all low-grade serous carcinomas arise from a preexisting serous borderline tumor, and it is possible that some or many do not. In contrast, high-grade serous carcinoma is not related to serous borderline tumor and was thought until recently to arise directly from the ovarian surface epithelium or the epithelium of cortical inclusion cysts with no well-defined precursor lesion. There is now emerging and quite compelling evidence (discussed later) that many high-grade ovarian serous carcinomas actually originate from the epithelium of the distal fimbrial portion of the fallopian tube [34–39]. As discussed, instead of grading ovarian serous carcinoma using a three-tiered system (well, moderate, poor; grade 1, 2, or 3), there is now a growing tendency among pathologists and oncologists to classify these as high grade or low grade and this practice has been adopted in the United Kingdom [24]; anecdotally many gynecological pathologists outside the United Kingdom now also use this two-tiered grading system which was originally proposed by the MD Anderson Group (Houston, Texas, USA). The classification of a serous carcinoma as low grade or high grade has been shown to be reproducible among pathologists [40, 41]. Almost all serous carcinomas which would have previously been classified as moderately or poorly differentiated represent high-grade neoplasms, while those which would have been classified as well differentiated may be either low grade or high grade using the two-tiered classification. For example, some architecturally well-differentiated serous carcinomas have high nuclear grade and represent examples of high-grade serous carcinoma. Although representing two distinct tumor types, on rare occasions a low-grade serous carcinoma component may coexist with and probably transform into a high-grade serous carcinoma, an undifferentiated carcinoma, or a carcinosarcoma, or a high-grade serous carcinoma may arise directly from a serous borderline tumor [42–44]. However, most low-grade serous carcinomas when they recur do so as low-grade neoplasms. Some advocate the term invasive micropapillary serous carcinoma as an alternative to low-grade serous carcinoma, but this terminology is not recommended since some low-grade serous carcinomas do not exhibit a micropapillary architecture and conversely many high-grade serous carcinomas do so. Figure 8.2 illustrates the postulated pathways of development of low-grade and high-grade serous carcinoma.

Molecular Events in Low-Grade and High-Grade Ovarian Serous Carcinoma

The underlying molecular events differ between low-grade and high-grade serous carcinoma. Low-grade serous carcinoma is associated with k-ras or b-raf mutation in approximately two-thirds of cases [25–31]. These mutations occur early in the evolution of low-grade serous carcinoma since they are also found in borderline and benign areas within the same neoplasm. K-ras and b-raf mutations appear mutually exclusive; in other words one, but not both, may be present in a particular neoplasm. The ERBB2 gene is also frequently mutated [31]. Low-grade serous

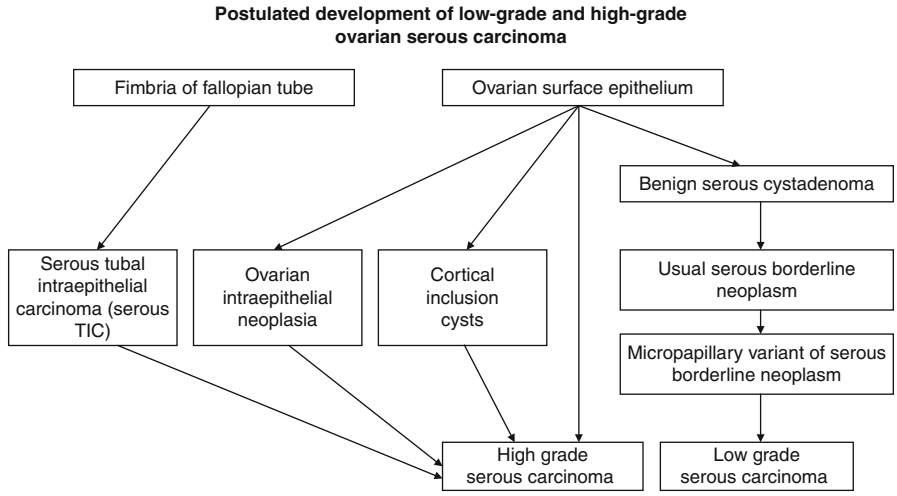

Fig. 8.2 Postulated developmental pathways of low-grade and high-grade serous carcinoma

carcinoma is not associated with TP53 mutations [25–31]. In contrast, high-grade serous carcinomas harbor, in most cases, a TP53 mutation or exhibit p53 dysfunction, and this appears to occur early in neoplastic development [25–31, 45]; they are not, except in occasional cases, associated with k-ras, b-raf, or ERBB2 mutation. One study, using stringent techniques, showed TP53 mutations to be ubiquitous in ovarian high-grade serous carcinomas with 97 % of these neoplasms exhibiting mutations; most of the mutation-negative cases exhibited TP53 dysfunction illustrating that p53 is abnormal in almost 100 % of ovarian high-grade serous carcinomas [46]. Consistent with this, high-grade serous carcinomas almost always exhibit aberrant p53 staining, most commonly diffuse intense nuclear immunoreactivity but sometimes totally negative staining ("all or nothing" staining). The latter type of immunoreactivity is usually due to nonsense TP53 mutation or deletion, in contrast to missense mutation which results in diffuse staining [47, 48].

High-grade serous carcinomas are also associated with BRCA1 and BRCA2 abnormalities, including germ line and somatic mutations, loss of heterozygosity, and epigenetic events such as promoter hypermethylation. For example, one study found germ line BRCA1 or BRCA2 mutation in 25 % of an unselected population of patients undergoing surgery for non-mucinous carcinomas of the ovary, fallopian tube, or peritoneum [49]. Over half of the patients with germ line mutation had no family history of ovarian carcinoma and would not have been referred for genetic testing. This raises the question as to whether all patients with pelvic high-grade serous carcinoma should be referred for BRCA genetic testing; this is currently the practice in the Ontario region of Canada. In another study, 47 % of ovarian high-grade serous carcinomas or undifferentiated carcinomas (the extreme end of the spectrum of high-grade serous carcinoma) had germ line or somatic BRCA1 mutations or epigenetic loss of BRCA1 [50].

Behavior of Low-Grade and High-Grade Ovarian Serous Carcinoma

Most low-grade and high-grade serous carcinomas are stage III or IV when diagnosed. At present, in most cases management is similar between these two tumor types, although there are exceptions. For example, adjuvant chemotherapy is not usually administered for a stage I low-grade serous carcinoma. Additionally, there is a growing realization among oncologists that low-grade serous carcinomas do not respond well to traditional chemotherapeutic agents, and if the tumor

is optimally debulked with no gross residual disease, some oncologists do not administer adjuvant chemotherapy even for advanced-stage low-grade serous carcinoma. Few studies have directly compared the outcome between low-grade and high-grade serous carcinomas. In one study, the survival of patients with low-grade neoplasms was significantly better than that of patients with high-grade tumors [40]. In this study, the 5-year survival for high-grade serous carcinoma was 9 % with a median survival of 1.7 years, while low-grade serous carcinomas had a median survival of 4.2 years and a 5-year survival of 40 % [40]. In another study of low-grade serous carcinomas, the median overall survival with stage III or IV disease was 6.8 years [51]. Low-grade serous carcinomas often prove fatal, but in some cases the course is indolent and protracted, and some patients survive for a considerable period of time, for example, in excess of 10 or 20 years.

Evidence for Origin of High-Grade Pelvic Serous Carcinoma from the Distal Fallopian Tube

There is recent convincing and accumulating evidence that many currently classified high-grade serous carcinomas of the ovary, fallopian tube, and peritoneum (collectively referred to as high-grade pelvic serous carcinoma) are derived from the fimbria of the fallopian tube [34–39]. Much of the initial evidence for this came from prophylactic salpingo-oophorectomy specimens in patients with germ line BRCA1 or BRCA2 mutation, these patients having a high risk of developing ovarian high-grade serous carcinoma. In initial studies, small high-grade serous carcinomas were occasionally identified in the ovary but this was rare. However, when pathologists began examining the fallopian tubes in their entirety, it was found that there was more likely to be a small in situ or invasive high-grade serous carcinoma involving the mucosa of the fimbria of the fallopian tube; the in situ lesions are referred to as serous tubal intraepithelial carcinoma (STIC). This is relatively uncommon but is occasionally seen in prophylactic salpingo-oophorectomy specimens in patients with BRCA1 or BRCA2 germ line mutation. Further studies systematically examined the fallopian tubes in patients with sporadic high-grade ovarian serous carcinoma and found similar lesions involving the fimbria in a significant percentage of cases [34, 37]. Furthermore, identical TP53 mutations were demonstrated within the ovarian and tubal lesions. While this does not unequivocally prove that the origin is within the fallopian tube (this could represent a tumor originating within the ovary and spreading to the fallopian tube), there is accumulating evidence that many high-grade pelvic serous carcinomas arise from the tubal fimbria from STIC. A p53 signature has also been demonstrated in the fallopian tube [52]. This takes the form of small foci of intense p53 nuclear staining involving consecutive secretory cells, most commonly but not exclusively in the fimbria, in the absence of morphological changes. TP53 mutations have been demonstrated in some p53 signatures, and these may represent the earliest stage of development of high-grade pelvic serous carcinoma. However, p53 signatures are extremely common in the fallopian tube even in patients with benign disease and no hereditary predisposition to developing ovarian cancer, and it is clear that only a small proportion will ever develop into a STIC. STIC is not diagnosed on the basis of p53 staining unless associated morphological alterations are present associated with a high MIB1 proliferation index. This is important since it has been shown that there is significant interobserver variability, even among specialist gynecological pathologists, in the diagnosis of STIC on hematoxylin and eosin-stained sections [53].

It is probable that not all high-grade pelvic serous carcinomas are derived from the fallopian tube. One study which systematically examined all of the fallopian tubes in a consecutive series of EOCs identified STIC in nearly 60 % of high-grade serous carcinomas but not in other morphological subtypes of EOC [37]. It is possible that some high-grade serous carcinomas do arise from the ovarian surface epithelium or the epithelium of cortical inclusion cysts, the latter

being commonly lined by ciliated epithelium identical to that lining the fallopian tube. Another possibility in those cases in which no premalignant or malignant lesion is found in the fallopian tube is that tubal epithelium may exfoliate and become incorporated into the ovary and subsequently give rise to a high-grade serous carcinoma [37]; in fact, it is possible that cortical inclusion cysts lined by ciliated epithelium are of tubal origin.

It can be summarized that there is accumulating evidence that many, or even most, high-grade pelvic serous carcinomas (high-grade serous carcinomas which are currently classified as ovarian, tubal, or peritoneal in origin) arise from the fimbria of the fallopian tube. In most cases, the malignant cells exfoliate readily from the fimbria into the pelvis and abdomen and result in the formation of an ovarian mass or masses usually, but not always, with disease elsewhere in the pelvis and abdomen; this is conventionally referred to as ovarian high-grade serous carcinoma. In other cases, the neoplasm remains localized to the fallopian tube, resulting in a fallopian tube high-grade serous carcinoma, or gives rise to extensive peritoneal disease in the absence of significant ovarian or tubal involvement; this is conventionally referred to as primary peritoneal high-grade serous carcinoma. It is likely that neoplasms which are currently classified as high-grade serous carcinomas of the ovary, fallopian tube, and peritoneum are all different manifestations of the same disease, and the designation high-grade pelvic serous carcinoma may be more appropriate. A consequence of these observations is that screening programs for ovarian carcinoma may be relatively ineffective in downstaging high-grade serous carcinomas since these are most likely disseminated from the outset. However, screening may be of value in picking up serous carcinomas when the burden of disease is lower and will also identify other morphological subtypes of carcinoma which largely remain localized within the ovary. Future studies investigating the underlying molecular events in the development of high-grade pelvic serous carcinoma should concentrate on the distal fallopian tube.

Evidence for Origin of Low-Grade Serous Carcinoma from the Fallopian Tube Epithelium

As discussed in the last section, there is mounting evidence that many or most so-called ovarian high-grade serous carcinomas arise from the epithelium of the distal fallopian tube. There is also some evidence that ovarian serous borderline tumors and low-grade serous carcinomas may also originate from the epithelium of the fallopian tube, although the evidence at present is much less in amount and less convincing than for high-grade serous carcinoma. It has been proposed that ovarian and extraovarian low-grade serous proliferations (low-grade serous carcinomas, serous borderline tumors, noninvasive implants, and endosalpingiosis) are derived from a lesion which has been referred to as papillary tubal hyperplasia in one study and which may be induced by chronic inflammation [54, 55]. Papillary tubal hyperplasia is characterized by small rounded clusters and papillae of tubal epithelial cells, with or without associated psammoma bodies, that are present within the tubal lumen. This is often seen in association with ovarian serous borderline tumors, although this does not prove that it is the origin of low-grade serous proliferations.

Mucinous Carcinoma

Primary ovarian mucinous carcinomas affect a wide age range, including occasionally children and adolescents. As discussed, they are relatively uncommon, the two population-based studies referred to earlier suggesting that they account for approximately 3 % of primary EOCs [10, 11], a significantly lower percentage than in older studies. In some institutions, the prevalence is higher for the reasons discussed earlier. The underlying reasons for the marked decline in primary ovarian mucinous carcinomas are well known. In older studies, it is clear that many presumed primary ovarian mucinous carcinomas, especially of advanced stage, were metastases from extraovarian sites. Advances in imaging,

serum markers, and preoperative workup have resulted in better recognition of metastatic ovarian neoplasms with the result that many of these are not surgically removed. Moreover, pathologists are now better at recognizing the morphological features of metastatic mucinous carcinoma in the ovary [1, 56–64], including the tendency to cystic change and the well-known "maturation phenomenon" with paradoxical better differentiation resulting in areas resembling benign and borderline mucinous cystadenoma. The use of differential cytokeratin staining and other immunohistochemical markers [64–72] has also improved the situation, although problems still exist. It is now clear that ovarian mucinous neoplasms associated with pseudomyxoma peritonei are almost always of appendiceal origin [73–75], with the very rare exception of primary ovarian mucinous neoplasms of overtly large intestinal type arising in a teratoma [76]. It seems to be an overtly large intestinal-type mucinous epithelium which has the potential to result in pseudomyxoma peritonei. There has also been a redefinition of the criteria for diagnosis of a well-differentiated mucinous carcinoma with so-called expansile (nondestructive, confluent glandular) invasion [1, 56, 58, 61]; as discussed, the distinction between this and a mucinous borderline tumor at the upper end of the spectrum still represents a somewhat poorly reproducible area among pathologists resulting in some variation in the reported prevalence of primary ovarian mucinous carcinoma between centers.

Most primary ovarian mucinous carcinomas are unilateral and stage I (occasionally stage II), and advanced-stage neoplasms (stage III or IV) are uncommon. In this scenario, a secondary should always be strongly considered. Although metastatic mucinous carcinomas in the ovary are still sometimes misdiagnosed as a primary ovarian mucinous carcinoma or even a mucinous borderline tumor due to the pronounced maturation effect seen with some secondary mucinous carcinomas in the ovary, we have to some extent come full circle in that there is now a tendency to overplay the possibility of a metastasis even when the pathological features are obviously those of a primary ovarian neoplasm. In a large majority of cases, the distinction between a primary and secondary mucinous carcinoma in the ovary can be achieved by careful pathological examination encompassing both the gross and microscopic findings and taking into account the distribution of the disease. It has been stated that when a mucinous carcinoma is diagnosed in the ovary, further investigations, such as colonoscopy and detailed imaging of the upper abdomen, should be undertaken to exclude a primary neoplasm elsewhere but this is unnecessary in most cases. Features suggesting a metastatic mucinous carcinoma in the ovary have been extensively discussed elsewhere [1, 56–64] and in Chaps. 10 and 16, and are listed in Table 8.5.

Table 8.5 Pathological features favoring metastasis in ovarian mucinous carcinoma[a]

Bilateral tumors
Small tumors
Nodular pattern of ovarian involvement
Macroscopic and microscopic surface deposits of tumor
Marked lymphovascular space invasion (especially outside ovary and in hilum)
Marked variation in growth pattern from one nodule to another
Infiltrative (destructive) stromal invasion
Single-cell infiltration and signet ring cells
Cells "floating" in mucin
Extraovarian spread

[a]None of these features are pathognomonic, but they are often seen in combination

Two Types of Primary Ovarian Mucinous Carcinoma

Most primary ovarian mucinous carcinomas (and borderline tumors) are of so-called intestinal (enteric or nonspecific) type. While many of these contain goblet cells, and even occasionally Paneth or neuroendocrine cells, the presence of goblet cells is not a prerequisite for an intestinal-type mucinous tumor. In fact, with regard to their mucin histochemical profile, many of these more closely resemble gastric or pancreatobiliary (upper gastrointestinal) mucinous neoplasms

[77]. A much more uncommon Mullerian (endocervical) variant of ovarian mucinous carcinoma and borderline tumor also exists [78, 79]. While borderline mucinous neoplasms of Mullerian type are well described, Mullerian mucinous adenocarcinomas are extremely uncommon and may, in the main, represent endometrioid carcinomas with marked accumulation of intracytoplasmic mucin.

Ovarian mucinous neoplasms of intestinal type comprise a spectrum or continuum from benign through borderline to malignant. In other words, intestinal-type ovarian mucinous carcinomas, like low-grade serous carcinomas, are thought to arise through a well-defined adenoma-carcinoma sequence from a benign cystadenoma through a borderline tumor to a mucinous carcinoma [1, 60]. Categories of intraepithelial carcinoma and microinvasion are also described. The designation intraepithelial carcinoma should be reserved for borderline tumors in which there is severe atypia of the epithelial lining [60]. Microinvasion is not uncommon in mucinous borderline tumors. The upper size limit has varied between studies but most use 5 mm (others use 3 mm or 10 mm^2); multiple separate areas of microinvasion may occur. Because of the continuum from benign to malignant, it is not uncommon to see an admixture of different morphological patterns (benign, borderline, borderline with intraepithelial carcinoma, microinvasion, carcinoma) side by side within an individual neoplasm. Given this heterogeneity, and the fact that primary ovarian intestinal-type mucinous neoplasms are typically very large, extensive pathological sampling is mandatory to rule out a small focus of invasion which may potentially result in adverse behavior. The degree of sampling necessary has been discussed previously [1], and it is generally recommended that one block per cm should be taken from tumors 10 cm or smaller and 2 blocks per cm of neoplasms larger than this. However, a degree of common sense should also be applied, and areas which are solid or different in appearance should be preferentially sampled, and if there are worrisome features in the initial sections, such as intraepithelial carcinoma or areas suspicious of invasion or microinvasion, additional sections should be examined.

Intestinal-type ovarian mucinous neoplasms are typically diffusely positive with CK7 but also commonly express, focally or diffusely, enteric markers such as CK20, CDX2, CEA, and CA19.9 and are negative with hormone receptors, CA125 and WT1 [1, 5]. CA19.9 especially is often diffusely positive, and there may be elevation of the serum level of this marker [80]. Serum CA19.9 levels may be extremely high and are of no value in predicting preoperatively whether an ovarian mucinous neoplasm is benign, borderline, or malignant [80].

Pathogenesis of Primary Ovarian Mucinous Carcinomas

In comparison to the other main subtypes of EOC, there has been less research into the pathogenesis of primary ovarian mucinous carcinomas. Similar to low-grade serous carcinomas, ovarian mucinous tumors of intestinal type commonly exhibit k-ras mutations, and identical mutations have been demonstrated in benign, borderline, and malignant areas within the same neoplasm, suggesting that k-ras mutation is an early event in the evolution of these tumors [81, 82]. Unlike low-grade serous carcinomas, b-raf mutations are not a feature of ovarian mucinous neoplasms of intestinal type. It has been generally assumed that primary ovarian mucinous neoplasms have arisen from the ovarian surface epithelium or the epithelium of cortical inclusion cysts through a process of mucinous metaplasia, but pathological evidence for this is lacking, and it is uncommon to see mucinous metaplasia of the ovarian surface epithelium or of cortical inclusion cysts. It is clear that some primary ovarian mucinous neoplasms of intestinal type arise from underlying teratomatous neoplasms. The exact proportion of ovarian mucinous neoplasms which arise in this manner is unknown since underlying teratomatous elements may become obliterated and overgrown by the mucinous neoplasm. Mullerian mucinous borderline tumors and carcinomas are often

associated with and probably arise from endometriosis and commonly exhibit k-ras mutations [83]. They may also exhibit ARID1A mutations like other neoplasms which arise in endometriosis [84].

It has recently been speculated that some primary ovarian mucinous neoplasms arise from transitional epithelium which is commonly seen in the paraovarian and paratubal tissues and in the fallopian tube mucosa, especially at the junction of the distal fallopian tube and peritoneum [84–88]. At the latter site, this is traditionally called transitional metaplasia and in the paraovarian and paratubal location, Walthard's rests; these represent the same basic lesion consisting of small nests of transitional epithelium. Evidence for transitional epithelium being the origin of some ovarian mucinous neoplasms includes the fact that small cystic foci lined by mucinous epithelium are commonly seen in ovarian Brenner tumors which have also been speculated to arise from transitional epithelium or Walthard's rests and the combination of Brenner tumor and mucinous neoplasm, especially mucinous cystadenoma, is not uncommon. It may be that analogous to other junctional epithelia, for example, the squamocolumnar junction in the uterine cervix, that the epithelium at the junction of the distal fallopian tube and peritoneum (so-called fallopian tube-peritoneal junction), whether it be distal tubal epithelium or transitional epithelium, is a junctional hotspot for neoplastic transformation.

Behavior of Primary Ovarian Mucinous Carcinomas

As discussed previously, most primary ovarian mucinous carcinomas of intestinal type are unilateral and stage I. Advanced-stage (stage III or IV) primary ovarian mucinous carcinomas are very rare, and a secondary should always be excluded. The prognosis of stage I primary ovarian mucinous carcinoma is relatively good, although some cases recur, usually in the pelvis or abdomen; recurrence is associated with a poor prognosis. In one population-based study of 31 primary ovarian mucinous carcinomas, 8 of 31 recurred [89]. Infiltrative (destructive) stromal invasion is associated with a worse prognosis than expansile invasion. Advanced-stage primary ovarian mucinous carcinomas have a dismal prognosis and respond poorly to the traditional chemotherapeutic agents used to treat ovarian carcinomas. Malignant mural nodules in ovarian mucinous neoplasms are traditionally thought to have a poor prognosis, although one large study has shown that stage 1A tumors with malignant mural nodules may be associated with a relatively favorable outcome [90].

Endometrioid Adenocarcinoma

Most, but not all, ovarian endometrioid adenocarcinomas are low grade and low stage (usually confined to the ovary – stage I). High-grade endometrioid adenocarcinomas exist but are uncommon. Occasionally there is an admixture of low-grade (grade 1 or 2) endometrioid adenocarcinoma and undifferentiated carcinoma, so-called dedifferentiated endometrioid carcinoma (this is discussed in the section Undifferentiated Carcinoma). Endometrioid adenocarcinomas are usually unilateral, but approximately 10 % are bilateral. They often, although not always, arise from endometriosis or a preexisting borderline adenofibroma [91–93]. The prevalence of primary ovarian endometrioid adenocarcinoma is lower in recent than in older studies [10, 11]. This is almost certainly due to the recognition that many neoplasms which were previously diagnosed as high-grade and high-stage endometrioid carcinomas are, in fact, serous in type. This is an area where previously there was poor reproducibility among pathologists and where WT1 staining may be useful (discussed later). With an endometrioid adenocarcinoma involving the ovary, there is not uncommonly a synchronous endometrioid proliferation, either premalignant or malignant, within the uterine corpus [93]. If conservative management (unilateral salpingo-oophorectomy) is undertaken for a stage I ovarian endometrioid adenocarcinoma, the endometrium should be sampled to exclude significant pathology. The prognosis of primary ovarian endometrioid adenocarcinomas is

extremely good since most are low grade and low stage at diagnosis.

Although less extensively studied, endometrioid adenocarcinomas of the ovary exhibit similar molecular events to those seen in uterine endometrioid adenocarcinomas; these include PTEN, β-catenin, k-ras and PIK 3CA mutations, and microsatellite instability [94]. β-Catenin mutations are especially characteristic of low-grade endometrioid adenocarcinomas with squamous morules [95]. ARID1A mutations have also been described [96, 97].

Clear Cell Carcinoma

Recent studies suggest that primary ovarian clear cell carcinomas occur with approximately equal frequency to endometrioid adenocarcinomas and are probably slightly more prevalent [10, 11]. Most clear cell carcinomas are diagnosed at early stage (stage I or II) and the majority arise in endometriosis. Careful pathological sampling, especially concentrating on cystic areas, will usually reveal background endometriosis, often in the form of an endometriotic cyst.

As discussed, it is recommended that ovarian clear cell carcinomas are automatically categorized as grade 3 [24]. Since these neoplasms are often architecturally well differentiated and sometimes have a relatively low cytological grade and are typically mitotically quite inactive (average 3–4 mitoses per 10 high-power fields [98]), formal grading using one of the universal systems may result in these being categorized as grade 1 or 2.

It is widely assumed that clear cell carcinomas have a relatively poor prognosis. However, the prognosis of stage I ovarian clear cell carcinomas is relatively good. Advanced-stage clear cell carcinomas have a poor prognosis and appear relatively resistant to the traditional chemotherapeutic agents used in the treatment of ovarian carcinoma; it is possible that this is because these neoplasms exhibit a low proliferation index. Ongoing trials are investigating the value of various chemotherapeutic agents in these neoplasms in comparison with traditional ovarian-based chemotherapy.

The underlying molecular events in ovarian clear cell carcinoma have not been extensively investigated [99], but ARID1A mutations have been demonstrated in a significant percentage of cases [96, 100, 101].

Transitional Carcinoma

Primary ovarian transitional carcinomas are rare, although they do exist [102]. Most tumors which are diagnosed as transitional carcinoma probably represent variants of high-grade serous carcinoma, and transitional carcinoma is a poorly reproducible diagnosis. Other tumors diagnosed as transitional carcinoma may represent endometrioid adenocarcinomas with a transitional-like growth pattern. Transitional carcinomas of the ovary express Mullerian and not urothelial markers and are usually positive with WT1 [103], a point in favor of many being variants of high-grade serous carcinoma. Some recur or metastasize as high-grade serous carcinoma, and this is further evidence that many are variants of the latter.

Undifferentiated Carcinoma

The WHO definition of an undifferentiated ovarian carcinoma is a primary ovarian carcinoma with no differentiation or only small foci of differentiation [9]. These are uncommon but not rare and most probably represent the extreme end of the spectrum of high-grade serous carcinoma since undifferentiated areas are not uncommonly seen in the latter. With a seemingly undifferentiated ovarian carcinoma, further sampling may reveal areas more diagnostic of high-grade serous carcinoma, such as vague papillary formations, slit-like spaces, or psammoma bodies. Global gene expression studies support the hypothesis that most undifferentiated ovarian carcinomas are related to high-grade serous carcinoma [3]. WT1 staining also supports this since many, but not all, undifferentiated ovarian carcinomas exhibit nuclear staining with this marker which is commonly expressed in ovarian serous carcinoma [1, 3]. It is reasonable to assume that an

undifferentiated carcinoma which is diffusely positive with WT1 represents the extreme end of the spectrum of high-grade serous carcinoma and to state this on the pathology report. A smaller number of undifferentiated ovarian carcinomas may represent dedifferentiation within a low-grade endometrioid adenocarcinoma; the concept of dedifferentiation within uterine and ovarian endometrioid adenocarcinomas, especially the former, has been highlighted in recent years [104].

Mixed Carcinomas

According to the WHO, a mixed carcinoma should only be diagnosed when the minor component makes up at least 10 % of the neoplasm [9]. However, all morphological subtypes within an ovarian carcinoma should be documented and the percentages listed, even if the minor component accounts for less than 10 %. True mixed carcinomas of the ovary (unlike in the uterus) are relatively uncommon, although they do occur [1–6]. A combination of endometrioid and clear cell carcinoma occasionally occurs since both tumor types commonly arise in endometriosis. Neoplasms which are diagnosed as mixed serous and endometrioid or mixed serous and clear cell carcinoma mostly represent high-grade serous carcinomas with areas which mimic endometrioid or clear cell carcinoma; the combination of serous and endometrioid or serous and clear cell carcinoma is uncommon. This is discussed further in the next section. The combinations of serous and undifferentiated or endometrioid and undifferentiated carcinoma have already been discussed. The former should be reported as a high-grade serous carcinoma with a comment that undifferentiated areas are present and that this is in keeping with the spectrum of high-grade serous carcinoma.

Problematic Areas in Typing of EOCs

The situation with regard to typing of ovarian carcinomas has improved over the past few years, and one study has shown that when using modern diagnostic criteria, excellent interobserver agreement can be achieved among specialist gynecological and general pathologists in the typing of EOCs [105]. In fact, the situation is much better with ovarian than uterine carcinomas where considerable problems still exist in the categorization of "high-grade" carcinomas. Many of the previous (and current) problems in classification of EOCs relate to the categories of high-grade serous, high-grade endometrioid, and undifferentiated carcinoma in which there is morphological overlap. Another problematic area is the categorization of clear cell areas within an ovarian carcinoma, specifically whether these represent a clear cell carcinoma or component of clear cell carcinoma or clear cells within a serous, endometrioid, or undifferentiated carcinoma.

Previously some pathologists tended to diagnose many poorly differentiated ovarian carcinomas as serous in type, while others classified them as endometrioid or mixed serous and endometrioid. The prevailing view is that the vast majority represent high-grade serous carcinomas. In this distinction, WT1 immunohistochemical staining may be of value [103–106]. Most (80–90 %) primary ovarian (as well as primary peritoneal and tubal) serous carcinomas exhibit diffuse nuclear positivity with WT1, while most endometrioid adenocarcinomas are negative or at the most focally positive [106–109]. In problematic cases, WT1 staining is recommended as an adjunct to help distinguish between a high-grade serous and a high-grade endometrioid adenocarcinoma. As discussed, most undifferentiated ovarian carcinomas represent the extreme end of the spectrum of high-grade serous carcinoma, and WT1 staining may be useful in this regard. In support of the now widely held view that most high-grade serous and undifferentiated carcinomas and what some pathologists still report as high-grade endometrioid adenocarcinomas represent variants of high-grade serous carcinoma are global gene expression studies which cannot distinguish these at a molecular level [3].

Characteristically in ovarian clear cell carcinoma, an admixture of growth patterns is present with tubulocystic, papillary, and solid architectures and eosinophilic stromal hyalinization [98]. Most clear cell carcinomas are diagnosed without

difficulty, but there is a tendency to overdiagnose clear cell carcinoma or a clear cell carcinoma component due to the presence of clear cells within serous and to a lesser extent endometrioid adenocarcinomas. The presence of areas of more typical serous or endometrioid adenocarcinoma is a useful pointer in diagnosis (as discussed, sometimes a combination of clear cell and endometrioid carcinoma occurs), and it is stressed that the mere presence of clear cells does not constitute a clear cell carcinoma. WT1, ER, and p53 are usually negative in ovarian clear cell carcinoma (p53 exhibits "wild-type" staining with a focal, weak, and heterogenous pattern); most high-grade serous carcinomas are positive with these markers, while most endometrioid adenocarcinomas are ER positive. Recently, hepatocyte nuclear factor 1 beta has been shown to be a promising nuclear marker of ovarian (and uterine) clear cell carcinomas [110, 111], although this is not a specific marker, and further studies are needed to more fully evaluate the full range of immunoreactivity with this marker.

Concept of Type I and Type II EOC

Given the recent major advances in our knowledge regarding the pathogenesis, natural history, and behavior of the main subtypes of EOC, it is now tempting to think of a broad dualistic pathway of ovarian epithelial carcinogenesis; similar to the uterus, the terms type I and type II ovarian carcinoma have been proposed [2], although this has been criticized and has not gained widespread acceptance. Certainly the terms type I and type II carcinoma should not replace the specific morphological subtypes, but like the dualistic pathway relating to the pathogenesis of uterine carcinomas, this terminology is concerned with broad mechanisms of tumor development. Type I tumors are considered to arise via a well-defined adenoma-carcinoma sequence from a benign precursor lesion, such as a borderline tumor or endometriosis, and to evolve in a stepwise fashion. Type I tumors are, in general, slow growing and indolent neoplasms and include low-grade serous, endometrioid, mucinous, and clear cell carcinoma and malignant Brenner tumor. There are obvious parallels with type I endometrial cancers which are also, in general, indolent and arise from a well-defined precursor, atypical hyperplasia. In contrast, type II ovarian carcinomas are high-grade clinically aggressive neoplasms. Most represent high-grade serous carcinoma. Carcinosarcoma (analogous to the situation in the uterine corpus, most ovarian examples represent high-grade serous carcinomas with sarcomatous elements which derive from the epithelial malignancy [112, 113]) and undifferentiated carcinoma, which are both predominantly variants of high-grade serous carcinoma, are also included in this category. Type II carcinomas are often associated with TP53 mutations, and there is emerging evidence that many arise from the epithelium of the distal fallopian tube. Again there are obvious parallels with type II endometrial cancers which are often associated with TP53 mutation. In rare cases, as discussed, a type I carcinoma can transform into a type II [42–44].

Do Primary EOCs Exist?

As has been discussed in this chapter, there is now ample evidence that many so-called ovarian high-grade serous carcinomas arise from the epithelium of the distal fallopian tube from a precursor known as STIC. There is also weaker but emerging evidence that low-grade serous carcinomas may also arise from the epithelium of the fallopian tube. It is clear that many ovarian endometrioid and clear cell carcinomas arise from endometriosis which is ultimately probably, in most cases, derived from the endometrium secondary to retrograde menstruation. Furthermore, it has been speculated that some ovarian mucinous neoplasms arise from the nests of transitional epithelium at the distal fallopian tube-peritoneal junction. Thus, there is evidence (stronger for some tumor types than others) that many or perhaps the majority of so-called primary EOCs are actually derived from extraovarian tissues [85]. While this may seem somewhat fanciful, it should be borne in mind that primary epithelial neoplasms of the testis are uncommon, most malignancies which arise in this organ being of germ cell or sex cord-stromal type.

Hence it is possible that, analogous to the testis, most primary ovarian malignancies are of germ cell or sex cord-stromal lineage.

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Serous Neoplasms of the Ovary

9

Eugenia C. Colón and Joseph W. Carlson

Abstract

Our understanding of the biology and clinical behavior of serous neoplasms of the ovary has changed enormously over the past decade. It is now clear that although all serous tumors share a cell type reflecting tubal epithelium in various levels of differentiation, they appear to have different origins and distinct behaviors. Benign and borderline serous neoplasms, the most common variety, arise through a progressive accumulation of molecular defects and appear to arise in the ovary, possibly from endometriosis. High-grade serous carcinomas, in contrast, seem to arise, at least in part, on the epithelium of the distal fallopian tube.

Molecular Pathology

Although serous tumors share a similar cell type, these tumors can be divided into two separate groups, with distinct biological, clinical, and histological characteristics. Histological use of the two-tier grading system (described below) can accurately classify the majority of tumors into these two biological subtypes, although there are occasional exceptions. Characteristics of these tumor types are summarized in Table 9.1 [1].

Low-grade serous borderline tumors and low-grade serous carcinoma are united by defects in the kinase cascade involving RAS, RAF, and MEK [2]. They appear to develop as part of a defined progression whereby a subset of hyperplastic but non-clonal serous cystadenomas develop into clonal serous borderline tumors through the acquisition, in part, of either K-RAS or BRAF mutations [3, 4]. Certain borderline tumors then proceed to invasive low-grade serous carcinoma, as shown by studies demonstrating shared mutations in a subset of these tumors. Interestingly, recent work has demonstrated that high-stage low-grade serous carcinomas have a surprisingly low frequency of BRAF mutations [5]. Complete exome sequencing of low-grade

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Table 9.1 Characteristics of serous neoplasms of the ovary

Low-grade serous neoplasms (Type 1)
Cell morphology: cells resembling fallopian tube epithelium (secretory and ciliated)
Diagnoses: cystadenoma, borderline, and low-grade carcinomas
Putative origin: ectopic Mullerian epithelium, for example, cortical inclusion cysts, endosalpingiosis, metaplastic endometriosis, or ovarian surface epithelium
Biology: BRAF and K-RAS mutations, HOX11 gene expression
High-grade serous neoplasms (Type 2)
Cell morphology: dedifferentiated cells rarely showing secretory and ciliated differentiation
Diagnoses: high-grade carcinoma
Putative origin: distal fallopian tube (majority), ectopic tubal epithelium (minority)
Biology: p53 mutation, accumulation of DNA damage, BRCA loss

invasive serous carcinomas shows very few point mutations when compared with other epithelial tumors [6].

High-grade serous tumors appear to arise through a combination of p53 mutation and damage to homologous DNA repair pathways, especially loss of BRCA1 or 2. This leads to tumors that are characterized by chromosomal instability and aneuploidy, with large gains and losses of chromosomal material [1]. A proportion of high-grade serous carcinomas appear to arise on the fallopian tube. The identification of the fallopian tube as a site of origin is the result of careful analysis of risk-reducing salpingo-oophorectomy resections from women at high risk for ovarian cancer [7–9]. Complete evaluation of the fallopian tube and ovary reveals the presence of in situ tubal cancers predominantly on the fimbria. These cancers are often occult and are detected prior to development of advanced peritoneal spread. Subsequent investigations have demonstrated the presence of serous tubal intraepithelial carcinoma in resections from non-BRCA bearers, indicating that this model appears to explain sporadic tumor development as well. Cancer development on the fallopian tube appears to involve DNA damage; acquisition of mutations, including P53; and probably loss of functional DNA repair pathways, such as BRCA [10]. High-grade serous tumors typically do not show mutations in BRAF and K-RAS [11].

Benign Serous Tumors

Macroscopically, these tumors show regions that are fibrotic, cystic, and papillary, in varying proportion (Fig. 9.1a). The papillae are thick and stubby. Upon closer inspection, however, they will have a uniform appearance, and although they are multicystic, they will usually not show any of the small papillary and micropapillary structures that are seen in borderline and malignant tumors. Necrosis and hemorrhage are not seen, unless secondary to torsion.

Microscopically, these tumors are typically characterized by overgrowth of the stroma or epithelium. The epithelium is a simple, nonstratified epithelium showing cilia, but occasionally showing a less differentiated simple cuboidal appearance (Fig. 9.1b, c). The stroma can be fibrotic and can grow in thick papillary structures, either within cysts or upon the surface of the tumor (Fig. 9.1d). Some tumors show a more fibrous background with entrapped simple cysts lined by the characteristic tubal epithelium (Fig. 9.1e, f).

These tumors are named based on the degree of papillary, cystic, and fibrotic growth that they show. Thus, a predominately cystic tumor would be termed a serous cystadenoma. A mixed cystic and fibrous tumor would be termed a serous cystadenofibroma. At the simplest end of the spectrum is a unilocular serous cyst, which can be termed serous cyst (if the epithelium shows definitive serous differentiation, i.e., cilia) or simple (i.e., indeterminate) cysts (if the epithelium is cuboidal without clear-cut differentiation). At the more complex end of the spectrum, a borderline tumor should be considered if there is a complex architectural pattern and a more complex epithelial growth. Areas of borderline-like complex growth should consist of at least 10 % of the tumor for it to be considered a borderline tumor.

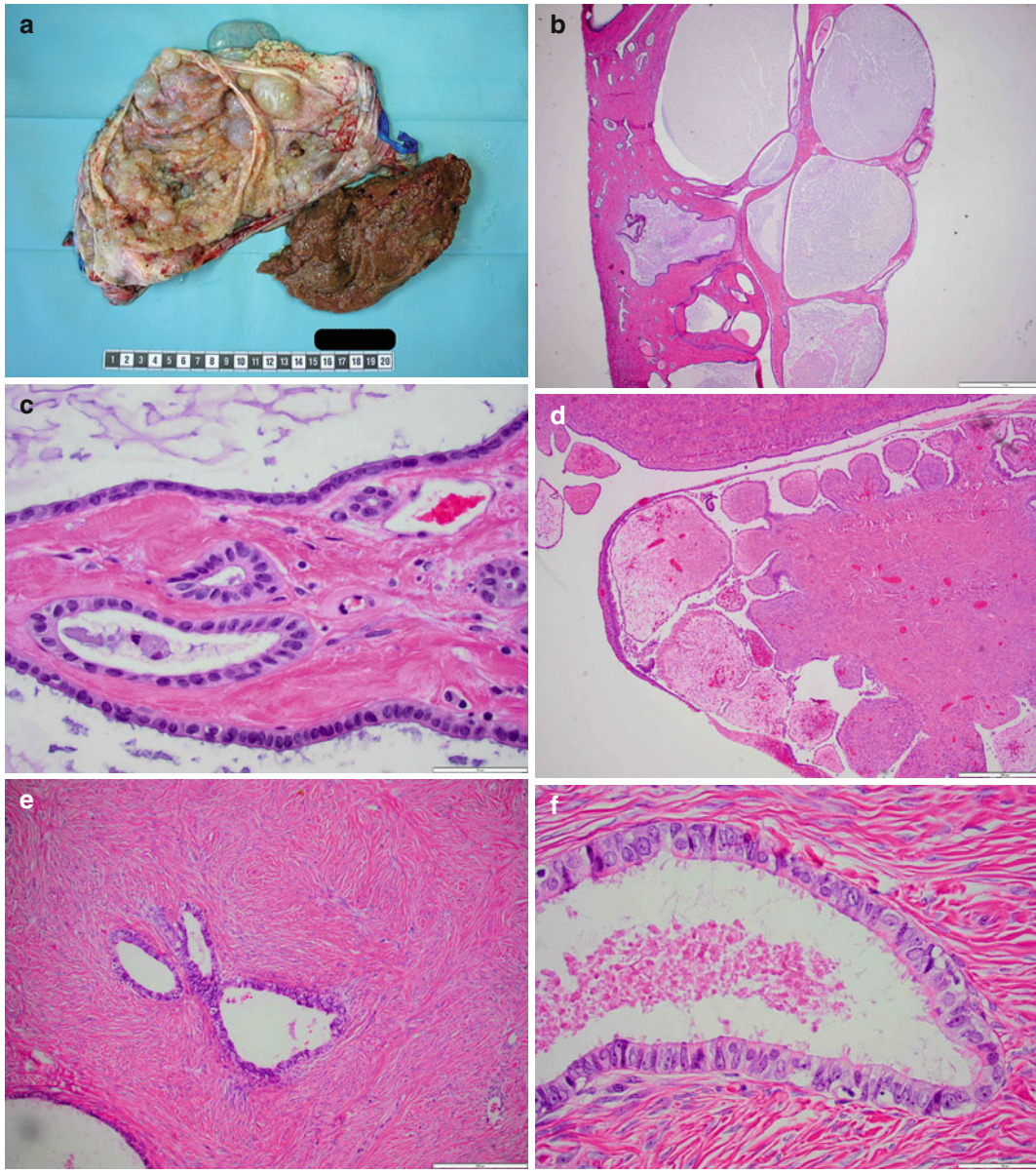

Fig. 9.1 Benign serous tumors. (**a**) Gross image of a serous cystadenoma. (**b**) Low-power image. (**c**) High power demonstrating ciliated serous epithelium. (**d**) Blunt papillae from serous papillary cystadenofibroma. (**e**) Low-power histology from a serous adenofibroma. (**f**) High-power view demonstrating benign serous epithelium

Finally, in terms of atypia, a benign serous tumor should show minimal cellular atypia. Areas with high-grade atypia, even if the cells are growing as a simple non-stratified epithelium, are not acceptable in a benign serous tumor. High-grade serous tumors can rarely grow in an "in situ" pattern, especially in the endometrium and fallopian tube fimbria, but we have seen cases with such growth on the ovarian surface, especially in resections from BRCA1 and 2 mutation carriers. Immunohistochemistry with antibodies against p53 will show strong nuclear staining, consistent with a mutated p53 gene, which is inconsistent with a benign serous tumor. In such

cases, additional tissue should be submitted for microscopic examination (including the entire ovary and fallopian tube).

Differential Diagnosis of Benign Serous Neoplasms

The differential diagnosis with benign lesions includes other cystic and fibrous lesions of the ovary and fallopian tube.

Endometriosis

This is distinguished by correct identification of the endometrial stroma cuffing the epithelium. Typically one can see hemosiderin deposition.

Cystically Dilated Follicles

These can be distinguished by correctly identifying two cell types lining the cyst (theca and granulosa cells). By tradition cysts less than 1 cm in diameter are typically termed cystic follicles, greater than 1 cm follicle cysts.

Hydrosalpinx

This can be mistaken for a benign serous tumor, especially if the macroscopic examination is performed without sufficient care. Histologically the fallopian tube is obviously lined by a serous epithelium, and the plicae in hydrosalpinx are typically attenuated. The fallopian tube, however, has an additional layer of smooth muscle not seen in the ovary.

Polycystic Ovarian Disease

The cysts of polycystic ovarian disease are follicular in nature, not serous. The diagnosis of polycystic ovarian disease is a clinical one, and while an ovary with multiple cysts may be suggestive of the disease, establishing the diagnosis requires clinical correlation (see Chap. 2).

Borderline Serous Tumors

Serous borderline tumors represent the prototypical borderline tumor in the gynecologic tract. These are tumors that, even when presenting at higher stages, can have a good prognosis and slow disease progression. This is in contrast to both low-grade and high-grade serous carcinoma. The terminology of the tumors is described in Table 9.2.

Table 9.2 Recommended and alternative terminology for serous borderline tumors

Recommended terminology	Alternative terminology
Serous borderline tumor	Atypical proliferative serous tumor
	Serous tumor of low malignant potential
Serous borderline tumor with micropapillary architecture	Noninvasive micropapillary serous carcinoma
	Low-grade serous intraepithelial carcinoma
Serous borderline tumor with microinvasion	Microinvasive carcinoma
Low-grade serous carcinoma	Low-grade (invasive) micropapillary serous carcinoma
Noninvasive epithelial peritoneal implant	Implant
Noninvasive desmoplastic peritoneal implant	Implant
Noninvasive implant with micropapillary pattern	Metastatic low-grade serous carcinoma
Invasive peritoneal implant	Metastatic low-grade serous carcinoma

Adapted from Refs. [21, 26, 33]

The central histological feature of serous borderline tumors is an architectural complexity to the serous epithelium, coupled with a mild increase in cytologic atypia. The majority of these tumors present with a large ovarian mass, and they are primary in the ovary. Rarely, these tumors can present with minimal to no ovarian involvement, and they are then primary in the peritoneum.

Macroscopically these tumors are characterized by the presence of numerous hierarchical papillary growths (Fig. 9.2a). These papillae can be either within the cyst walls of the tumor or on the ovarian surface. The papillae typically have a small to unidentifiable core, in contrast to the papillae of benign adenofibromas, where the papillae are often thicker and more "cauliflower-like." In contrast to malignant tumors, there is typically a lack of solid growth and necrosis,

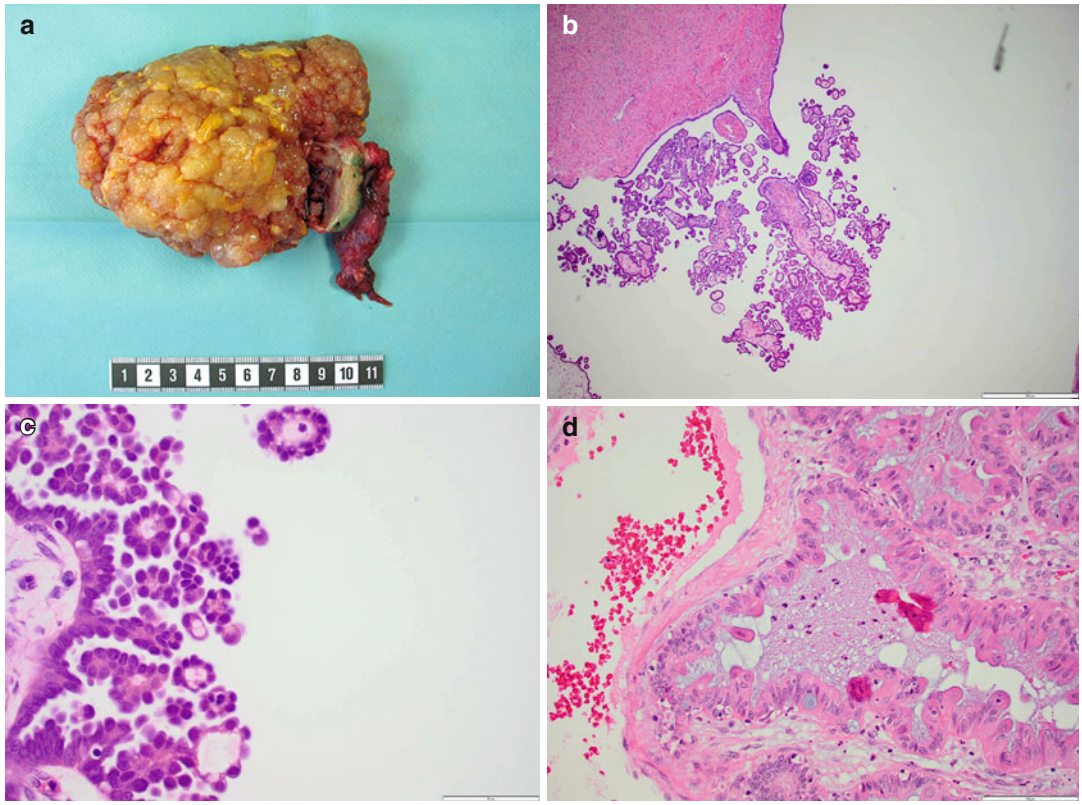

Fig. 9.2 Serous borderline tumor. (**a**) Gross image of a serous borderline tumor with exuberant growth on the external surface of the ovary. (**b**) Low-power histology of a serous borderline tumor showing the hierarchical papillary growth. (**c**) High-power histology showing the lack of cellular atypia. (**d**) Serous borderline tumor from a pregnant women showing eosinophilic cell change

although it should be emphasized that borderline and malignant tumors cannot be reliably distinguished using only macroscopic examination.

Upon macroscopic examination it is important to identify whether or not the ovarian surface is involved by the tumor. Both a carefully documented macroscopic examination (e.g., "a 2 cm area of suspect surface involvement is identified macroscopically") and the selective use of ink and microscopic examination are essential to satisfactorily demonstrate surface growth.

Cutting in of these tumors requires care because they can easily contaminate other specimens. We recommend cutting in these cases "out of order" by taking the tumor-free specimens first. For example, if the omentum is macroscopically uninvolved, then it would be cut in first. The ovarian tumors are cut in last, followed by a complete cleaning of the bench, a washing of instruments, and a changing of all knife and scalpel blades.

These tumors should be sampled to capture the most complex growth areas seen macroscopically. Areas of solid growth and necrosis should be particularly sampled. In cases where complex growth is minimal, the quantity of papillary growth should be estimated macroscopically to allow distinction from a serous cystadenoma (10 % is the lower cutoff for a serous borderline tumor, and it is very difficult to judge this using only microscopy without macroscopic correlation). At a minimum, one section per centimeter of diameter <10 cm, and 2 sections per centimeter of diameter >10 cm (excluding simple thin-walled cystic components), should be taken to exclude areas of microinvasion and frank invasion [12].

The typical low-power microscopic appearance is of complex papillae, the majority of which have a fibrovascular core (Fig. 9.2b). In contrast to cystadenomas, however, the papillae show hierarchical growth, with progressively smaller papillae, and the formation of micropapillae (i.e., papillae without a fibrovascular core) at their tips. One often encounters free-floating clusters of epithelial cells (Fig. 9.2c). Cytologic atypia should be of a low to moderate grade. High-grade cytologic atypia (i.e., where the atypia is equivalent to that seen in high-grade serous carcinoma) is incompatible with a diagnosis of a borderline tumor. Such high-grade atypia is usually associated with a mutation in p53, and so immunohistochemistry with p53 can be of assistance in this differential diagnosis. In pregnant women the tumor cells often have a more eosinophilic appearance by routine hematoxylin and eosin staining (Fig. 9.2d).

Micropapillary Architecture

The micropapillary pattern is characterized by long, thin micropapillae (i.e., lacking a fibrovascular core) that are at least five times longer than they are wide (Fig. 9.3a, b). This pattern is characterized by abrupt micropapillary growth, in what is called a "medusa head" pattern. This pattern should cover an area of 5 mm or more. This is in contrast to the usual borderline growth pattern, where large papillae give rise to medium size which in turn gives rise to small micropapillae. Abrupt micropapillary growth reflects the sudden change from large, blunt papillae to long,

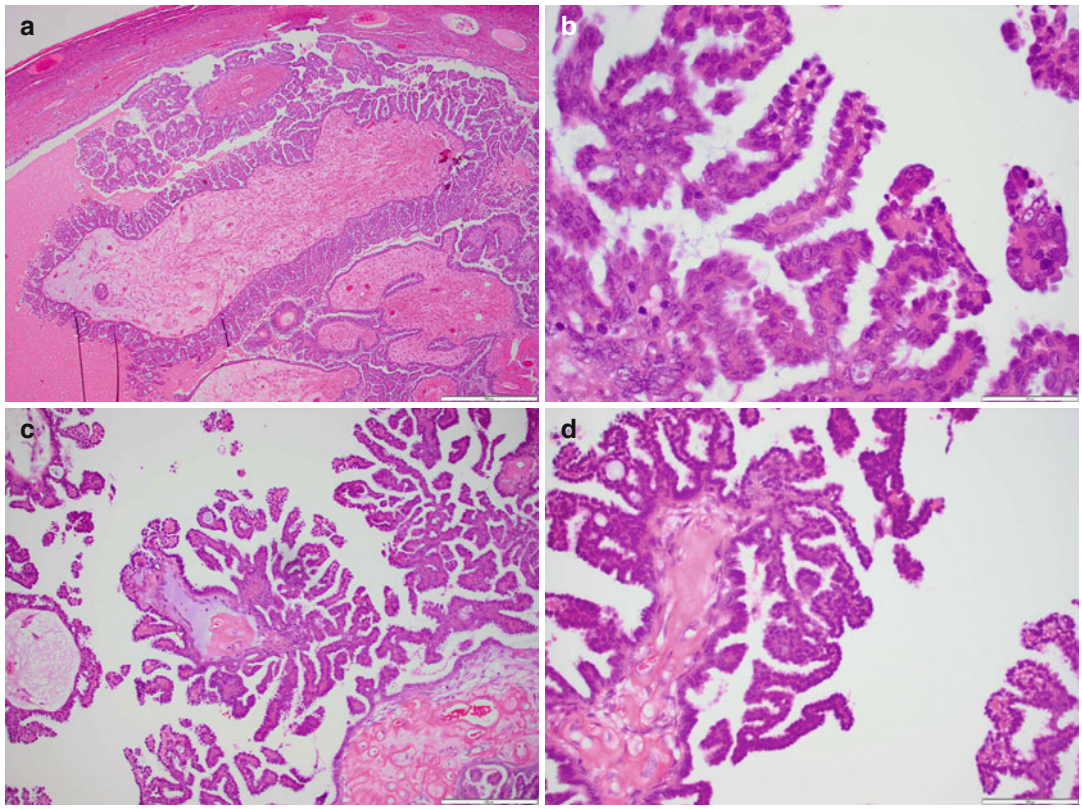

Fig. 9.3 Serous borderline tumor with micropapillary architecture. (**a**) Low-power image showing abrupt micropapillary "medusa head" pattern. (**b**) High-power image showing papillae that are roughly five times longer than they are wise. (**c**) High-power image showing the lack of cellular atypia. (**d**) Additional low-power image showing abrupt transition to long, thin papillae

thin micropapillae, without intervening intermediary sizes. Less common growth patterns that can be considered within this group include cribriform and almost solid growth of noninvasive cells on the outside of a papillary stalk.

There has been extensive investigation into the role of the micropapillary pattern since its first description in 1996 [13]. Multiple studies have indicated that serous borderline tumors with a micropapillary pattern have a worse prognosis than those without it [14–18]. However, the poor prognosis of the micropapillary pattern appears to be related to its strong association with invasive implants. Serous borderline tumors with a micropapillary pattern that are low stage or have noninvasive implants appear to have the same prognosis as serous borderline tumors without invasive implants. The micropapillary pattern, therefore, does not appear to be an independent prognostic marker, but instead appears to be associated with invasive peritoneal implants [19].

There is currently controversy regarding the correct terminology for borderline tumors showing micropapillary growth. Some authors have proposed terminology that includes "micropapillary serous carcinoma" and "serous borderline tumor with in situ carcinoma." Given the good prognosis of the majority of these tumors, we recommend continuing to name them as "serous borderline tumors" and indicating which of the above patterns is present (e.g., "serous borderline tumor with micropapillary architecture"), but avoiding the use of the term carcinoma. This is in keeping with other experts in the field [20].

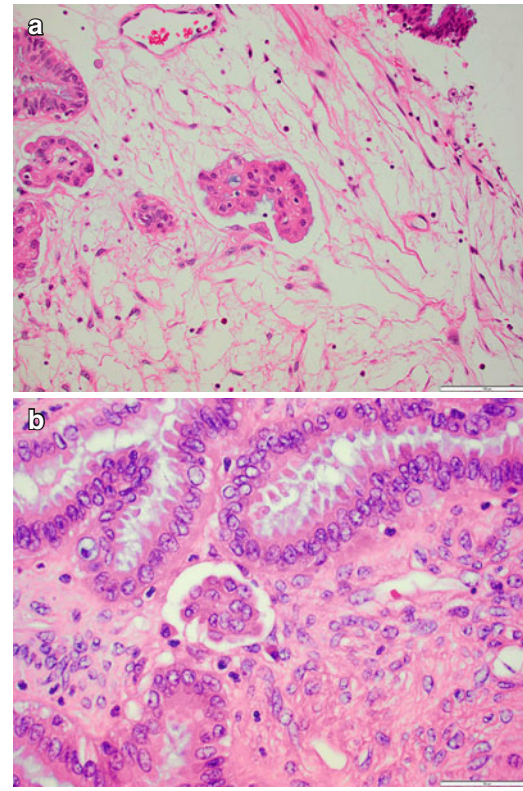

Fig. 9.4 Serous borderline tumor with microinvasion. (**a**, **b**) Small clusters of serous epithelium, typically with eosinophilic cell change, within the stroma, are typical of microinvasion. The focus or foci should measure less than 10 mm^2 or 3 mm in linear dimension

Microinvasion

Microinvasion is defined as the presence of small groups of cells or single cells invading the stroma of a borderline tumor (Fig. 9.4a, b). These small groups of cells often show an increased eosinophilia of the cytoplasm and a retraction of the surrounding stroma. Plane of section artifact should be excluded. The size of microinvasion has varied in the literature, but the WHO defines it as one or several foci, none of which exceeds a maximum linear dimension of 3 mm and a maximum area of 10 mm^2 [21, 22]. There is no limit to the number of foci that are allowed, and no definition of "multifocality" is given.

The presence of microinvasion should be reported, but it should not lead to the diagnosis of low-grade serous carcinoma if the tumor is otherwise characteristic of a borderline tumor. Microinvasion has not been found to be of independent prognostic significance, although it is often associated with other worrisome features, such as micropapillary growth pattern, involvement of the ovarian surface, and spread to lymph nodes. Clinical management of borderline tumors with microinvasion should be the same as borderline tumors without microinvasion; chemotherapy is not indicated in these cases.

Peritoneal Implants

Approximately 30 % of serous borderline tumors present at high stage, with spread of tumor within the peritoneum and to lymph nodes [23]. Approximately 60 % of these patients will have no disease recurrence. Since the prognosis is favorable for these patients, the term "implant," instead of "metastasis," has been used to designate foci of peritoneal spread. The nature of the peritoneal implants has been repeatedly shown to be a strong independent risk factor for tumor recurrence [24]. Thus, careful evaluation and correct interpretation of the nature of peritoneal implants is one of the most important tasks of the pathologist evaluating these tumors.

Peritoneal implants have been classified into invasive, noninvasive epithelial, and noninvasive desmoplastic. Noninvasive implants are divided into epithelial and desmoplastic types. Noninvasive implants can extend into the interlocular fibrous septa of the omentum without tissue invasion and destruction [21]. Epithelial-type noninvasive implants fill submesothelial spaces, demonstrate exophytic proliferations with hierarchical branching papillae, are composed primarily of epithelial cells, show no stromal reaction, and show frequent psammoma bodies (Fig. 9.5a, b) [21]. Desmoplastic-type noninvasive implants show a proliferation that appears plastered on the peritoneal surface, and they show nests of cells, glands, or papillae that proliferate in a prominent (>50 %) background of dense fibroblastic or granulation tissue with well-defined margins (Fig. 9.6a, b) [21].

The determination of invasion requires sampling of the underlying tissue. Invasive implants show haphazardly distributed glands invading normal tissue, loose or dense fibrous reaction without significant inflammation, a generally dominant epithelial proliferation, nuclear features resembling low-grade serous carcinoma, irregular borders, and they are often aneuploid (Fig. 9.7a–d) [21]. Invasive implants are those which show destruction of underlying normal tissue. Detection of tissue invasion is complicated by the fact that many biopsies have no normal underlying tissue for evaluation (up to 26 % in one study [25]). Some authors have proposed that additional criteria be used for identification of invasion in implants, including micropapillary growth and solid epithelial nests surrounded by clefts [25]. These patterns are so commonly associated with invasive implants that it has not been possible to validate their independent prognostic role. Fewer than 15 % of peritoneal implants are invasive.

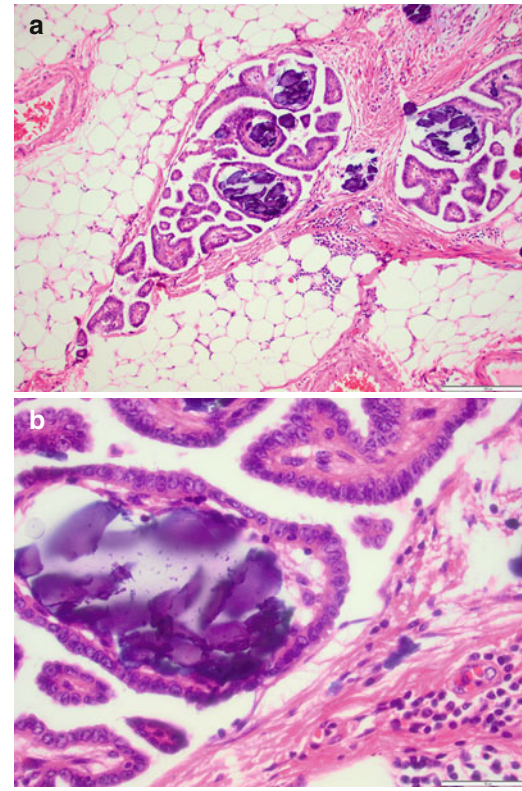

Fig. 9.5 Peritoneal noninvasive epithelial implants. (**a**, **b**) Clusters of serous epithelium in the interlocular fibrous septa of the omentum

In the absence of underlying tissue, the implant should be classified as noninvasive if the cytologic atypia is low to moderate and the architectural complexity is minimal. If the level of cytologic atypia resembles that of low-grade serous carcinoma or the architecture is complex, an implant lacking underlying tissue should be classified as indeterminate due to a lack of an evaluable tissue interface. The indeterminate category may also be used if the implant includes underlying tissue, but a categorical determination cannot be made

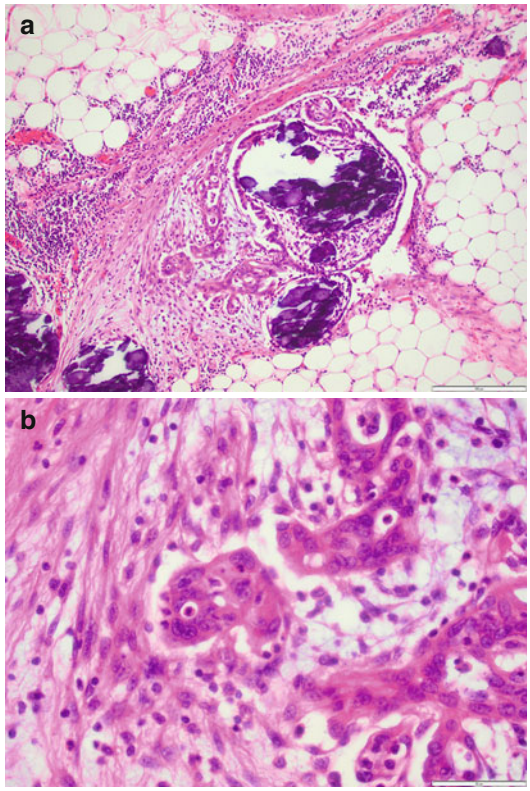

Fig. 9.6 Noninvasive desmoplastic implant. (**a**, **b**) Serous borderline epithelium involving the omentum with desmoplasia without underlying tissue destruction

regarding invasion (i.e., suspicious for invasion) [26]. The characteristics of peritoneal implants are summarized in Table 9.3.

Lymph Node Involvement

There are three types of lymph node involvement that should be identified and distinguished [12]. The first consists of epithelial inclusions with simple, non-stratified, tubal epithelium. These represent endosalpingiosis and are found in the capsule or fibrous septa. They are benign, although higher frequency of endosalpingiosis was associated with recurrence in a group of women with stage I borderline tumor [27].

The second type of lymph node involvement is the presence of clusters of cells, and even papillary fragments and growths, with the same morphologic appearance as the borderline tumor (Fig. 9.8a, b). These clusters of cells are typically seen within a glandular inclusion. These lesions do not appear to worsen prognosis. Both of the lesions mentioned above should be distinguished from mesothelial hyperplasia, which can lead to the presence of small clusters of mesothelial cells in lymph node sinus.

Finally, in some borderline tumors, lymph nodes may show areas of low-grade or high-grade invasive serous carcinoma. The key distinction here is the presence of tissue invasion in or around the lymph node. These tumors may arise in the lymph node, given that cases have been reported in the absence of an ovarian tumor [28].

Differential Diagnosis of Serous Borderline Tumors

Serous Cystadenoma

To establish the diagnosis of a serous borderline tumor, at least 10 % of the tumor should have areas of borderline-like proliferation. If the tumor has less than this, we diagnose the tumor as a serous cystadenoma with focal proliferation and explain in a comment that areas of borderline-like change are identified, but are insufficiently developed to make the diagnosis of a borderline tumor.

Low-Grade Serous Carcinoma

Areas of invasion in the primary tumor that are larger than microinvasion should lead to the diagnosis of low-grade serous carcinoma. In addition, low-grade serous carcinoma may show areas of solid or otherwise complex growth with marked epithelial proliferation that is inconsistent with a borderline tumor. Finally, low-grade serous carcinoma typically shows slightly greater cell atypia than that seen in a serous borderline tumor, although significantly less than in high-grade serous carcinoma.

High-Grade Serous Carcinoma

Serous borderline tumors should be inspected at high power to exclude the presence of high-grade cytologic atypia. Some high-grade carcinomas can mimic borderline tumors at low power (Fig. 9.10g).

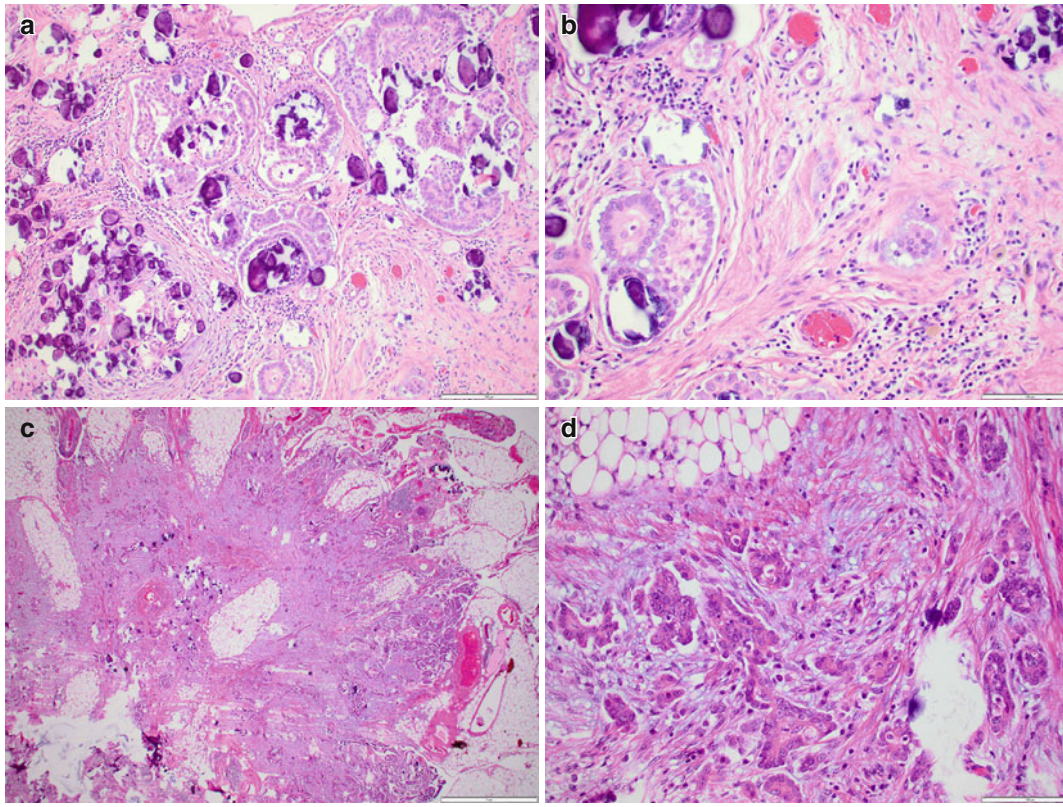

Fig. 9.7 Invasive peritoneal implant. (**a**–**d**) Serous epithelium involving the omentum with underlying tissue destruction

Table 9.3 Characteristics of peritoneal implants [21, 26]

Implant	Morphology
Noninvasive epithelial peritoneal implant	Extend into the fibrous septa of the omentum Hierarchical branching papillae No stromal reaction
Noninvasive desmoplastic peritoneal implant	Dense fibroblastic or granulation tissue background "plastered on" appearance *or* underlying tissue is lacking, low to moderate architectural complexity, atypia resembles a serous borderline tumor
Peritoneal implant, indeterminate for invasion	1. Biopsy lacks underlying normal tissue *and either* cytologic atypia clearly resembles low-grade serous carcinoma *or* the architecture is highly complex 2. Biopsy includes underlying normal tissue, but a categorical determination cannot be made (suspicious for invasion)
Invasive peritoneal implant	Haphazardly invading glands with irregular borders Predominately epithelial component

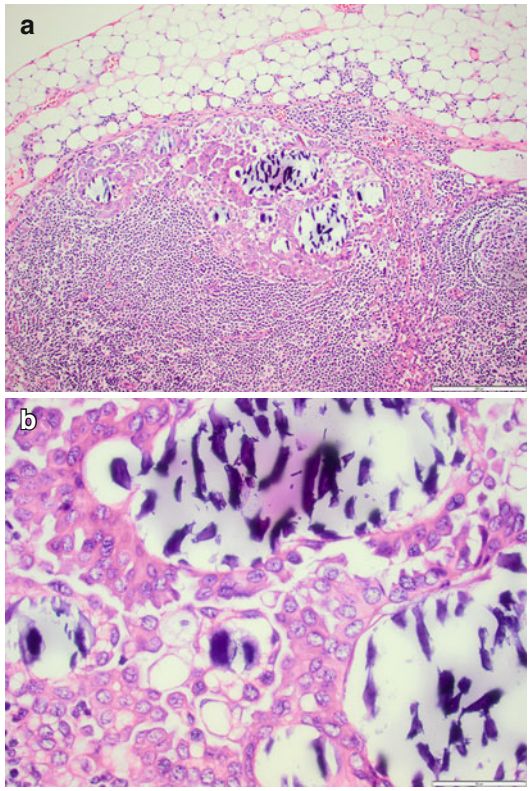

Fig. 9.8 Lymph node involvement by a serous borderline tumor. (**a**, **b**) Serous epithelium in the sinus of a lymph node from a patient with a borderline tumor

Mesothelial Lesions

Well-differentiated papillary mesothelioma can resemble a borderline tumor, but does not show areas of clear serous differentiation. The epithelium of these tumors is mesothelial, and so they are positive for calretinin. Malignant mesothelioma requires invasion and will be discussed in the differential diagnosis of high-grade serous carcinomas.

Struma Ovarii

Rare cases of struma ovarii can have a papillary architecture and can resemble a serous borderline tumor. Recognition of these tumors is usually not a challenge given that they contain colloid and the lining epithelium is columnar without tubal differentiation. Immunohistochemistry with TTF-1 would resolve any difficult case.

Table 9.4 Malpica two-grade system for serous neoplasms [29, 30]

The 2-tier grading system (high grade versus low grade) is based primarily on the assessment of nuclear atypia, with the mitotic rate used as a secondary feature
Low grade: a serous carcinoma composed of uniform cells with mild to moderate nuclear atypia and usually a low mitotic index (≤12 mitoses per 10 high-power fields [HPFs])
High grade: a serous carcinoma composed of pleomorphic cells with marked nuclear atypia (≥3:1 variation in size and shape) and a high mitotic index (>12 mitoses per 10 HPFs)

Retiform Sertoli-Leydig Cell Tumor

The epithelium of these tumors will not show tubal differentiation. These tumors are also inhibin and calretinin positive by immunohistochemistry.

Grading Invasive Serous Carcinomas

Several grading systems have been proposed for serous carcinomas, including WHO, Shimizu-Silverberg, FIGO, and the GOG grading system. As described above, serous carcinomas are actually two distinct tumor entities, with distinct clinical behavior and biology. A recent two-grade system specifically for serous carcinoma reflects this paradigm shift. The two-grade system, on multivariate analysis, has been shown to be a significant independent prognostic factor for overall survival. This system is highly reproducible and biologically relevant and is recommended by the Royal College of Pathologists in the UK [29, 30].

Grading with the two-grade system uses nuclear atypia as the primary criterion, with mitotic frequency as a second criterion. Low-grade serous carcinomas have uniform nuclei and a mitotic rate <12 mitoses per 10 high-power fields. High-grade serous carcinomas show either ≥3 times variation in nuclear size or >12 mitoses per 10 high-power fields. This grading system is summarized in Table 9.4.

Low-Grade Serous Carcinoma

Low-grade serous carcinomas have a similar macroscopic appearance to borderline and high-grade carcinomas. Cutting in of these tumors is similar to those tumors. Often extensive sampling is required to satisfactorily establish the invasive nature of the tumor.

The invasive component of low-grade serous carcinoma typically shows a more reliably papillary growth pattern than that of high-grade serous carcinoma, with the presence of micropapillae, macropapillae, small nests, and large nests of cells that show stromal infiltration, typically surrounded by a cleft or retraction artifact. The key to recognizing this pattern of infiltration is to note that the stromal spaces are not lined by serous epithelium as one would see with a section through a borderline tumor. The macropapillary pattern can be particularly difficult to recognize and can be confused with a serous adenofibroma [31].

Necrosis in low-grade serous carcinomas is uncommon. Sometimes psammoma bodies are so extensive that the epithelial component is almost indistinguishable. These tumors have been termed psammocarcinomas (Fig. 9.9a, b). As described above under grading, the nuclei typically show <3 times variation in size, and the mitotic rate is <12 per 10 high-power fields. Low-grade serous carcinomas can also show extensive cribriform growth (Fig. 9.9c, d).

Differential Diagnosis of Low-Grade Serous Carcinomas

Serous Borderline Tumor

Sometimes a serous borderline tumor will show an area of invasion that is larger than 10 mm^2. A tumor showing such a large focus of invasion should be classified as low-grade serous carcinoma arising in a serous borderline tumor.

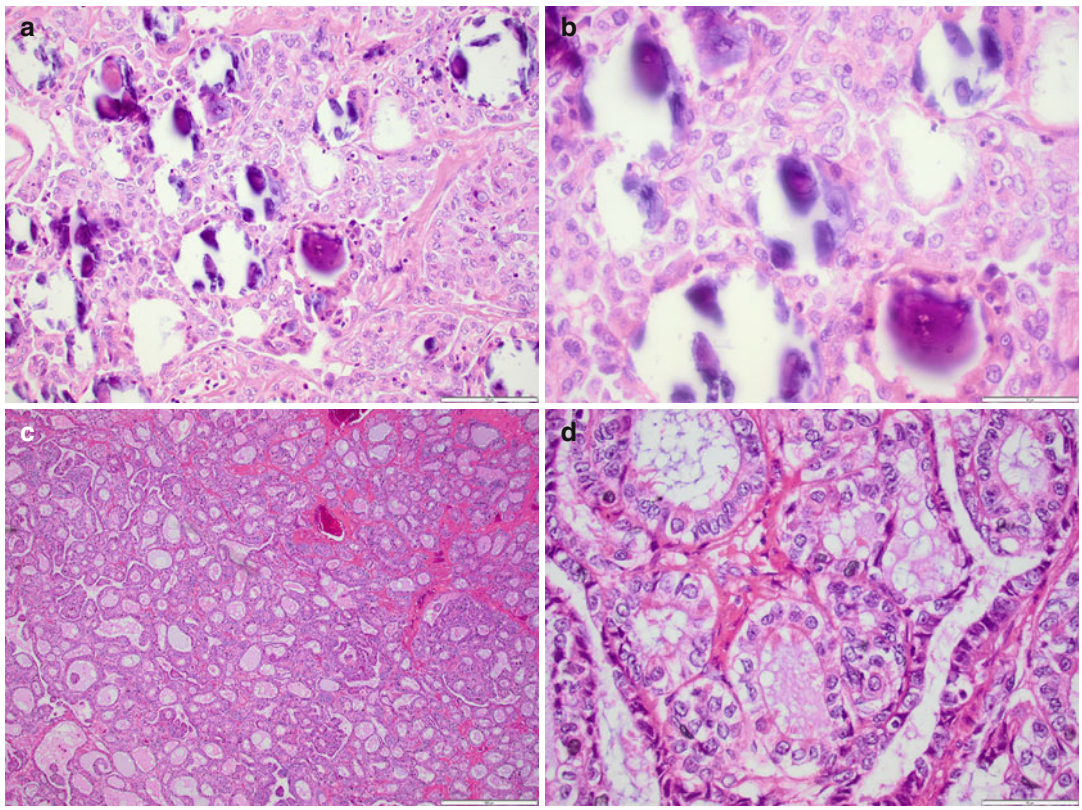

Fig. 9.9 Low-grade serous carcinoma. (**a**, **b**) Psammocarcinoma, a variant of invasive low-grade serous carcinoma. (**c**, **d**) Invasive low-grade serous carcinoma showing cribriform growth. The cells retain their cilia

Solid growth or extensive cribriform growth (not limited to lining papillary structures) can also lead to consideration of a low-grade serous carcinoma.

Malignant Mesothelioma

This will be discussed in the differential diagnosis of high-grade serous carcinoma.

High-Grade Serous Carcinoma

High-grade serous carcinomas are the most common type of ovarian carcinoma, constituting approximately 70 % of all ovarian carcinomas. The majority of patients present with advanced stage. These tumors show a wide range of histological appearances sometimes making an accurate diagnosis challenging, especially on biopsy material. As discussed previously under biology, high-grade serous carcinomas typically show p53 mutations and defects in DNA repair pathways. These tumors may derive from the fallopian tube epithelium (tubal intraepithelial carcinoma) or ectopic fallopian tube epithelium (endosalpingiosis) in a significant number of cases.

Macroscopically, high-grade serous carcinomas are typically large tumors with cystic, solid, and papillary areas. They can additionally show areas of solid growth and necrosis. The majority of these tumors present at high stage, and so the ovarian and peritoneal surfaces are often covered by tumor nodules (Fig. 9.10a). Sometimes these tumor metastases can be small and subtle.

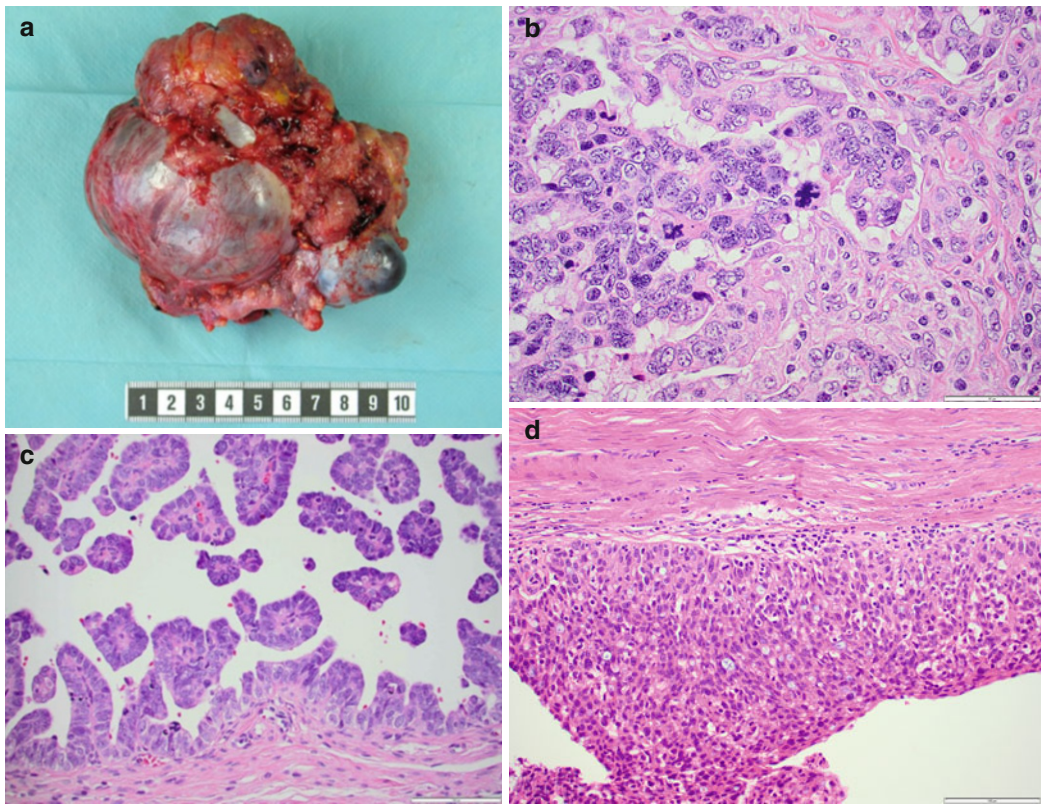

Fig. 9.10 High-grade serous carcinoma. (**a**) Macroscopic photo of a high-grade serous carcinoma. The fallopian tube is involved by tumor and is seen in the lower portion of the resection. (**b**) Tumor cells show high-grade atypia and abundant mitoses, including atypical forms. (**c**) Classic papillary growth. This growth pattern is not required for a diagnosis of high-grade serous carcinoma. (**d**) Stratified growth pattern that can resemble transitional cell carcinoma. (**e**) Microcystic growth. When high-grade atypia is present, these are best classified as serous and not endometrioid. (**f**) Clear cell change in a serous tumor should not warrant classification as a mixed tumor. (**g**) Areas that could resemble a borderline tumor. The nuclear atypia is inconsistent with a borderline of low-grade carcinoma. (**h**) Slit-like spaces are common in high-grade serous carcinoma

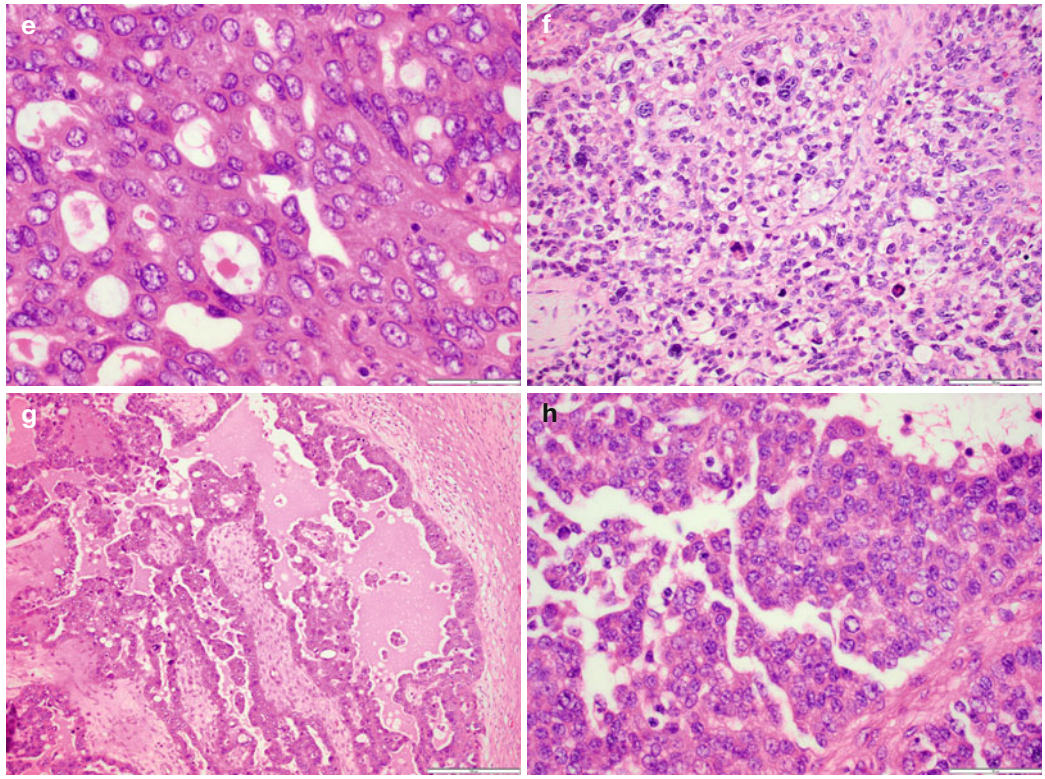

Fig. 9.10 (continued)

Cutting in of high-grade serous carcinomas is focused on evaluation of the ovarian surface for tumor growth (particularly important if the tumor has not spread), opening the tumor to ensure proper formalin fixation, and sampling the tumor sufficiently to allow correct diagnosis. These tumors are typically clearly malignant, so the number of sections required is often less than borderline tumors.

High-grade serous carcinoma shows a wide variety of growth patterns, with extensive variation within tumors and within sections (Fig. 9.10b–h). The most common is the presence of papillary and micropapillary growth, including tufts of malignant cells. These tumors can also show clefting and slit-like spaces. Solid and glandular patterns may also be seen. This solid pattern can occasionally raise concern for a dedifferentiated carcinoma, but in the presence of other areas characteristic of serous carcinoma, these tumors are best considered serous and not mixed. Glandular and microcystic patterns can raise concern for an endometrioid tumor, but, if the cytologic features are not consistent with an endometrioid tumor with corresponding tall columnar cells with low to moderate nuclear atypia, we consider this to be a variant growth pattern of serous carcinoma.

Some tumors can show a pattern which resembles high-grade urothelial carcinoma, which has earned them the name "transitional cell carcinoma." Studies have shown that this "transitional cell carcinoma"-like growth pattern has an immunophenotype similar to other high-grade serous carcinomas. Furthermore, these tumors lack the immunophenotype of urothelial cell carcinomas, indicating that they are best considered a variant of high-grade serous carcinoma. Clear cell change can also be observed, but this should not lead to the diagnosis of "mixed serous and clear cell carcinoma." The assignment of primary site is presented in Table 9.5.

Table 9.5 Assigning primary site in serous neoplasms

Modified system	
Tubal primary	Detection of serous tubal intraepithelial carcinoma (STIC), independent of the tumor burden on the ovary or peritoneum
Ovarian primary	Absence of STIC Invasive tumor in the ovarian stroma measures >5×5 mm
Primary peritoneal	Absence of STIC Tumor involves peritoneal surfaces Invasive tumor involves ovarian surfaces, invasion in the ovarian stroma measures <5×5 mm
Tubo-ovarian	Tumor has overgrown the adnexa so that the fallopian tube cannot be satisfactorily evaluated
Traditional system	
Tubal primary	Tumor must be macroscopically located in the tube or on the fimbriated end, without tumor on the ovary or peritoneum
Ovarian primary	Invasive tumor in the ovarian stroma measures >5×5 mm
Primary peritoneal	Tumor involves peritoneal surfaces Invasive tumor involves ovarian surfaces, invasion in the ovarian stroma measures <5×5 mm
Tubo-ovarian	Tumor has overgrown the adnexa so that the fallopian tube cannot be satisfactorily evaluated

Differential Diagnosis of High-Grade Serous Carcinomas

Low-Grade Serous Carcinoma

Occasional tumors can show areas with lower-grade atypia and fewer mitoses. Grading should be performed on the lowest differentiated portion of the tumor. With sufficient sampling and evaluation, the tumor grade should be apparent. Grading can be more challenging on peritoneal biopsy material prior to neoadjuvant chemotherapy, and in these cases care should be taken to exclude a low-grade or borderline tumor.

Endometrioid Carcinoma

Glandular and microcystic growth patterns can lead to consideration of an endometrioid carcinoma. However, high-grade nuclear atypia is not consistent with endometrioid differentiation. Also, areas of the tumor with more typical serous differentiation can usually be identified. Unless clearly distinct and obviously endometrioid (i.e., columnar epithelium with low to moderate atypia), a diagnosis of mixed carcinoma should be avoided, as these are extremely uncommon in the ovary.

Clear Cell Carcinoma

Areas of clear cell change should similarly not lead to a diagnosis of a mixed clear cell and serous carcinoma. Immunohistochemistry can help in this differential. Most serous carcinomas are positive with WT-1 and show aberrant p53 expression. Clear cell carcinomas are typically negative for these markers, as well as ER and PR. They are also positive for HNF-1(beta).

Malignant Mesothelioma

Primary peritoneal mesothelioma is extremely rare, but it can resemble serous carcinoma if care is not taken. Malignant mesothelioma typically has a different morphology than serous carcinoma, with a lack of pleomorphism and the high mitotic rate seen in serous carcinomas. Additionally, mesothelioma is positive for mesothelial markers, such as calretinin.

Metastatic Carcinoma

Serous carcinomas showing a solid growth pattern, particularly on small biopsy specimens, can raise the question of a metastatic poorly differentiated carcinoma. Immunohistochemistry can be used to confirm that the tumor has an immunophenotype consistent with serous carcinoma in difficult cases.

Immunohistochemistry of Serous Tumors

Immunohistochemistry can play an important role in excluding metastases and confirming the tumor as serous in type. Cytokeratin 7 is diffusely positive in ovarian carcinomas and negative in colon adenocarcinoma. Cytokeratin 20 shows a reverse profile. Serous ovarian tumors are typically diffusely positive with WT-1 and PAX-8.

WT-1 expression is not seen in, for example, breast, gastrointestinal, and pancreatic tumors. Estrogen and progesterone receptors are seen in approximately 40 % of primary ovarian tumors. CDX-2 is a specific marker for colon tumors, and DPC4 expression is lost in approximately 50 % of pancreatic carcinomas. The marker p16 is diffusely positive in high-grade serous carcinomas. The recommended panel for excluding metastases is therefore CK7, CK20, PAX-8, WT-1, GCDFP-15, CDX-2, DPC4, p16, and beta-catenin [32].

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Mucinous Neoplasms of the Ovary

10

Philip P.C. Ip and Annie N.Y. Cheung

Abstract

Primary mucinous tumors represent 15 % of all ovarian tumors and are classified as either benign, borderline, or malignant. More than 80 % of mucinous tumors are benign, while only 2–7 % are malignant. An overwhelming majority of tumors show gastrointestinal differentiation. Those of Müllerian differentiation are more commonly borderline tumors and rarely benign or malignant. Distinction between intestinal- and Müllerian-type carcinomas may not always be easy, owing to tumor heterogeneity and the fact that some tumors are mixed, such that components of benign, borderline, and carcinoma often coexist within an individual lesion. The diagnostic difficulty is further compounded by the fact that some tumors do not fit agreeably into the three biologic subcategories. Benign tumors containing <10 % of borderline features have been designated cystadenomas with focal atypia or focal proliferation. They are biologically benign. Borderline tumors have mild-to-moderate cytologic atypia, and almost all are followed by an uneventful outcome. In those with severe atypia and a complex architecture but without stromal invasion, the term mucinous borderline tumor with intraepithelial carcinoma is used. These latter tumors have a very low risk of recurrence. Microinvasion refers to borderline tumors, whether with or without intraepithelial carcinoma, that have ≥1 foci of tumor cells infiltrating the stroma but with each focus <10 mm^2. Provided the tumor has been adequately sampled to exclude occult foci of frank carcinoma, microinvasion does not seem to be an adverse prognostic factor. For mucinous carcinomas, the pattern of invasion is prognostically more relevant than grading, especially for stage I. Expansile-type stromal invasion refers to florid epithelial proliferation without intervening stroma. In infiltrative-type stromal invasion, there is irregular infiltration of the stroma by cells associated with stromal desmoplasia. The latter pattern is significantly associated with an adverse outcome.

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Molecular Pathology

The most common molecular change in mucinous tumors of the ovary is KRAS mutation at codon 12/13, involving >75 % of mucinous carcinomas and, to a lesser degree, their adjacent borderline and benign tumors. The latter supports the fact that there is a continuum of tumor progression from benign, borderline, to malignant and that KRAS mutation appears to be an early event in carcinogenesis [1–7]. Other early molecular events of crucial importance include p16 loss and RAS/RAF pathway alterations [8]. HER2 overexpression has been found in about 18 % of mucinous carcinomas and in 18 % of borderline tumors and is a potential target for therapy [9]. Tumors that showed both HER2 amplification and KRAS mutation have been shown to be prognostically favorable [9, 10].

Benign Mucinous Tumors

Synonyms

Mucinous cystadenoma

Clinical Features

Mucinous cystadenomas represent approximately 80 % of all ovarian mucinous tumors (Table 10.1). Some containing merely gastrointestinal epithelium may be of germ cell origin (monodermal teratoma). A minority may originate from Brenner tumors. The mean age of patients is 50 years and they usually present with a mass.

Gross Features

Mucinous cystadenomas are often unilateral and large, measuring 15–30 cm (mean, 10 cm) and weighing up to 4 kg [11]. They have a thick capsule and are either unilocular or multilocular (Fig. 10.1). The cyst wall may be either thin or thick and fibrous (adenofibromatous). Mucinous cystadenomas of Müllerian (endocervical-like) type are very rare. They are often uniloculated or have a few locules and do not have any grossly visible polypoid excrescences of a borderline tumor (see section "Endocervical-like mucinous borderline tumor"). They often contain altered blood or watery mucinous fluid. Some coexist with endometriosis.

Microscopic Features

The glands and cysts are usually separated by varying amounts of fibrous stroma, often with periglandular hypercellularity. Back-to-back

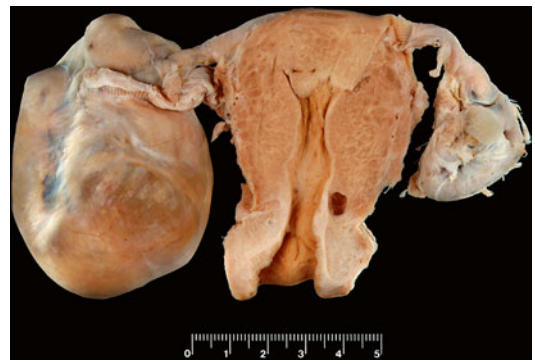

Fig. 10.1 Mucinous cystadenoma. The capsule is fibrous and thick

Table 10.1 Classification of ovarian mucinous tumors

Benign	Cystadenoma	
	Adenofibroma and cystadenofibroma	
Borderline	Intestinal type	*With microinvasion and/or intraepithelial carcinoma*
	Endocervical-like type (seromucinous)	
Malignant	Adenocarcinoma and cystadenocarcinoma	*With expansile invasion or with infiltrative invasion*
	Adenocarcinofibroma (malignant adenofibroma)	
	Mucinous tumor with mural nodules	
	Mucinous tumor with pseudomyxoma peritonei	

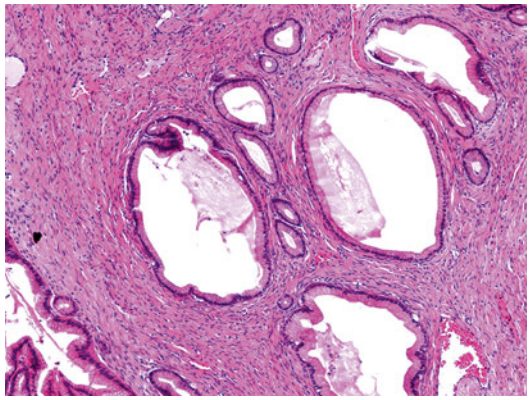

Fig. 10.2 Mucinous cystadenoma. The glands are surrounded by variable amount of fibrous stroma

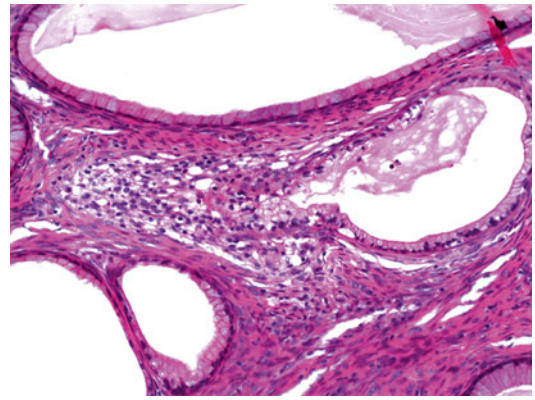

Fig. 10.4 Mucinous cystadenoma. Gland rupture with mucin granuloma

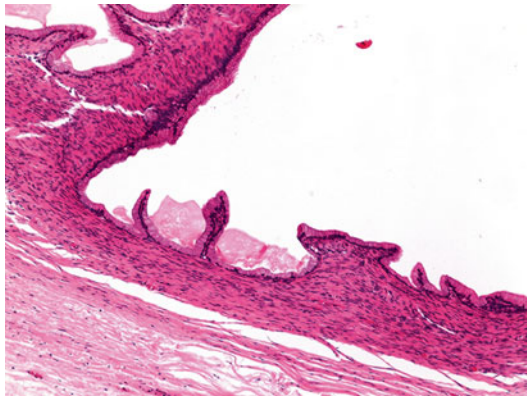

Fig. 10.3 Mucinous cystadenoma. Filiform papillae projecting into cystic space

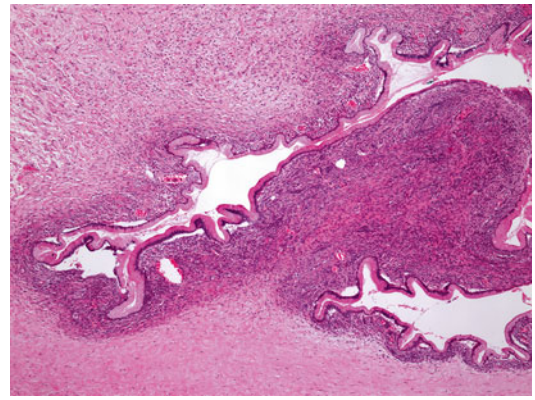

Fig. 10.5 Mucinous cystadenoma. The glands or cysts may be surrounded by a rim of condensed ovarian stroma

crowded glands may be seen, but there is almost always a thin rim of intervening stroma. The lining cells comprise a uniform layer of nonstratified columnar epithelium, with basally located nuclei and apical cytoplasmic mucin vacuoles. Almost all mucinous cystadenomas are of intestinal type. Although the "picket-fence" arrangement of the epithelium resembles that of endocervical mucosa, it has been noted that these epithelia more often show gastric pyloric differentiation (Figs. 10.2, 10.3, 10.4, 10.5, and 10.6) [11]. There should be no cytologic atypia. When there is mild-to-moderate atypia and nuclear stratification and tufting are present in mucinous borderline tumor, the designation of mucinous cystadenoma with focal atypia or focal proliferation may be used, provided the atypical component is <10 % of the entire tumor (Figs. 10.22, 10.23, 10.24).

The epithelium in mucinous cystadenomas of Müllerian (endocervical-like) type comprises single layer of cytologically bland mucous and eosinophilic cells. The latter may form epithelial tufts but true papillae with fibrovascular cores are not found. There is almost always striking inflammatory cell infiltration comprising neutrophils (Figs. 10.7 and 10.8).

Clinical Outcome and Prognosis

Mucinous cystadenomas are benign, even when there is focal atypia or focal proliferation (as defined above).

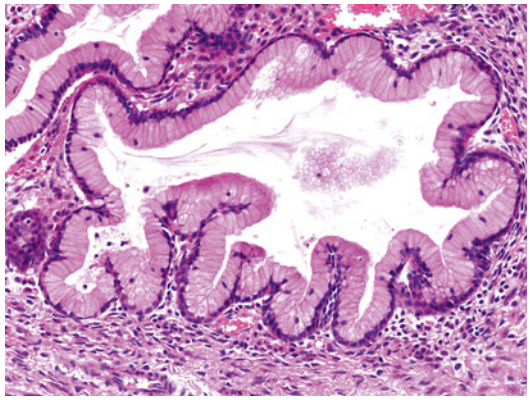

Fig. 10.6 Mucinous cystadenoma. The epithelial cells have basally located nuclei and apical mucin vacuoles

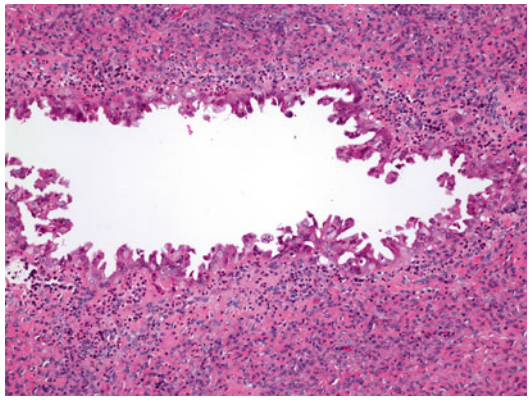

Fig. 10.7 Mucinous cystadenoma, endocervical-like. The epithelium may form tufts, but there is no intracystic papillary proliferation, in contrast to Fig. 10.25

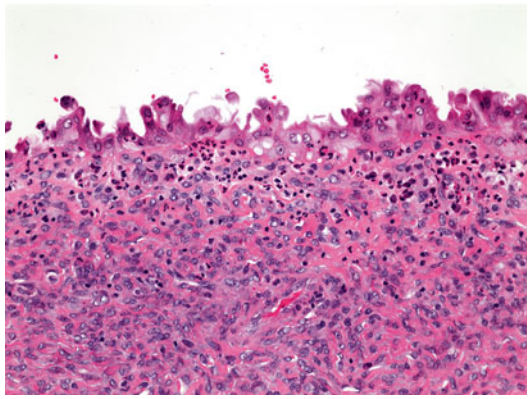

Fig. 10.8 Mucinous cystadenoma, endocervical-like. The lining epithelial cells comprise mucous cells and eosinophilic cells. Note prominent presence of inflammatory cells

Mucinous Borderline Tumors

Intestinal-Type Mucinous Borderline Tumors

Synonyms

(Intestinal-type) mucinous borderline tumor; Atypical proliferative mucinous tumor; Mucinous tumor of low malignant potential; Mucinous tumor of borderline malignancy

Clinical Features

Intestinal-type mucinous borderline tumors (IMBTs) usually affect women with a mean age of 51 years [11]. In the mucinous borderline tumor category, IMBT is the more common subtype and accounts for 85–90 % of cases. In >90 %, they are stage I, and 5–10 % are bilateral [12, 13]. When bilateral, a metastatic adenocarcinoma should always be excluded [14].

Gross Features

IMBTs are usually multiloculated and large, ranging from 18 to 22 cm and usually have a smooth and fibrous capsule (Figs. 10.9 and 10.10) [11].

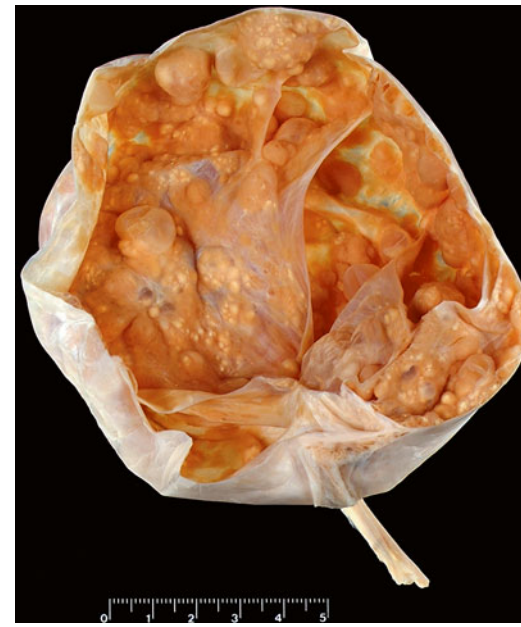

Fig. 10.9 Mucinous borderline tumor, intestinal type. Multiple internal locules of various sizes

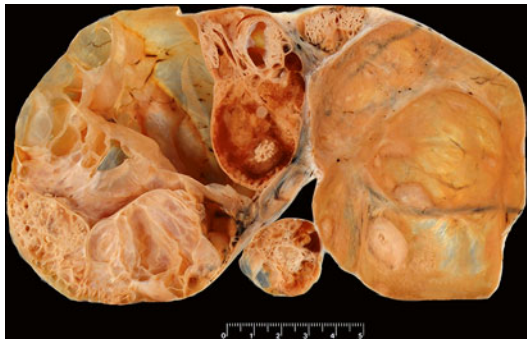

Fig. 10.10 Mucinous borderline tumor, intestinal type. Cross section of the small locules reveals even more internal loculations or septated cysts

The wall of the locules may be thin and translucent or may be thick and fibrous, rarely with polypoid excrescences. The locules usually contain clear and watery mucin but may be thick and viscous [1, 6, 15–18]. Hemorrhage and necrosis may be present and should not be interpreted as signs of malignancy. IMBTs are sometimes grossly indistinguishable from some benign cystadenomas or even carcinomas; generous sampling for microscopic examination is recommended. Although the general rule is to take 1 block for each centimeter of the tumor, it is equally important to note that this should be carried and focused on the solid areas, not just the thinner cyst lining.

Microscopic Features

The glands and cysts may be closely packed or widely separated by stroma. The glands may be fused, but true cribriform glands, i.e., those without any discernible stroma between individual glandular spaces, are infrequent. There are usually epithelial tufts or filiform papillae projecting into glandular spaces, but papillae with fibrovascular stromal cores, in particular those with hierarchical branching as encountered in serous borderline tumors, are not usually found. The cells lining the glands and cysts and those covering the filiform papillae are similar to those seen in a benign tumor but show mild-to-moderate pleomorphism (Figs. 10.11, 10.12, 10.13, 10.14, and 10.15). Goblet cells and, occasionally, Paneth cells and neuroendocrine cells are

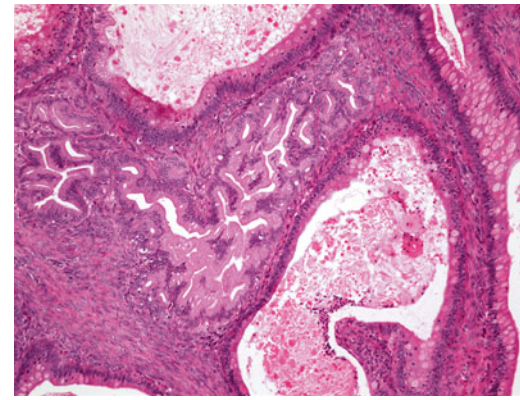

Fig. 10.11 Mucinous borderline tumor, intestinal type. Either the glands or cysts are lined by a single layer of epithelium, or there may be internal epithelial cross-bridges

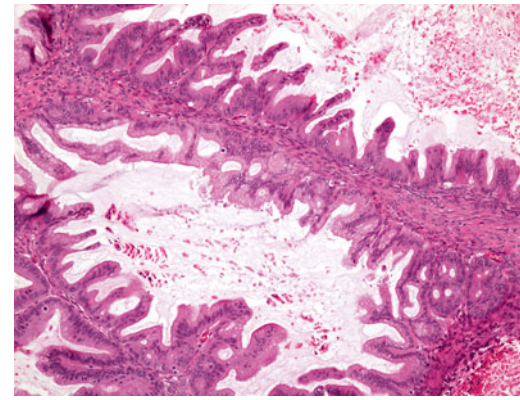

Fig. 10.12 Mucinous borderline tumor, intestinal type. Filiform papillae projecting into cystic spaces

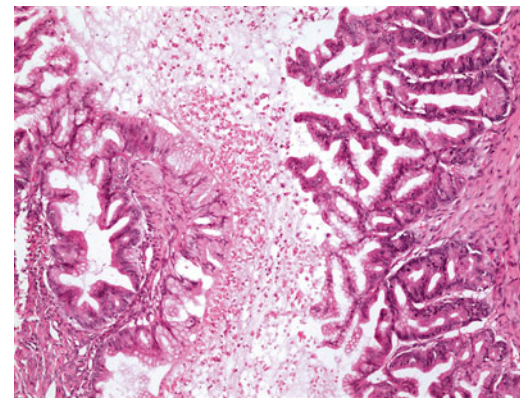

Fig. 10.13 Mucinous borderline tumor, intestinal type. Lining epithelium ranges from those resembling benign mucinous cystadenoma (*left*) to those that are architecturally complex and cytologically abnormal (*right*)

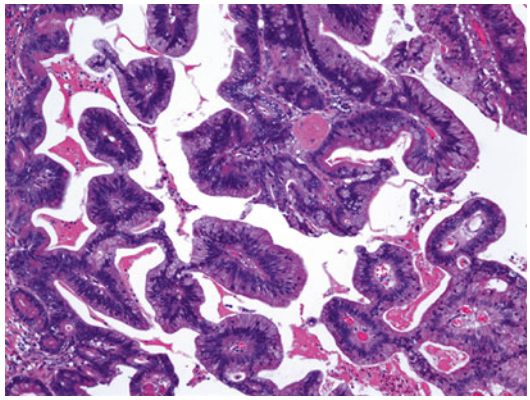

Fig. 10.14 Mucinous borderline tumor, intestinal type. Papillary pattern

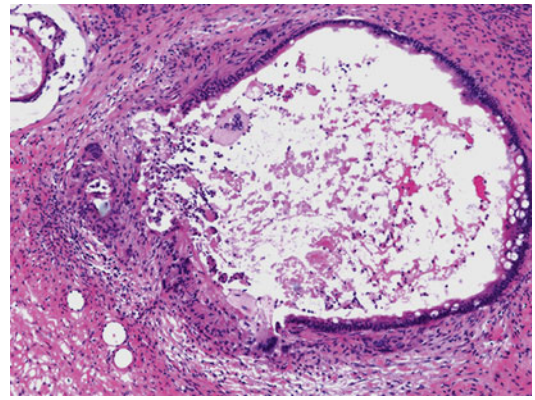

Fig. 10.16 Mucinous borderline tumor, intestinal type. A ruptured gland with mucin granuloma. This is sharply demarcated from the surrounding stroma

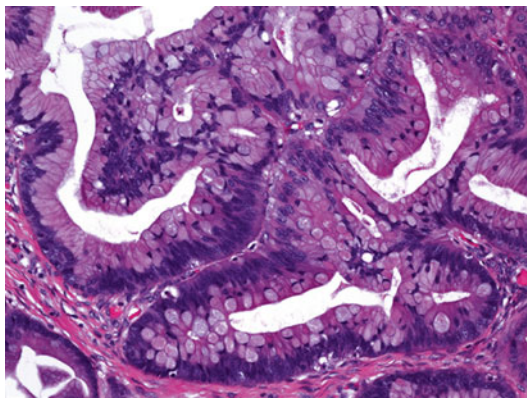

Fig. 10.15 Mucinous borderline tumor, intestinal type. The epithelium is enteric type, with prominence of goblet cells

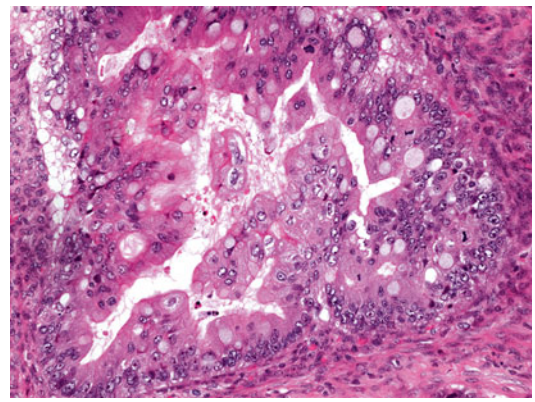

Fig. 10.17 Mucinous borderline tumor, intestinal type, with intraepithelial carcinoma. The epithelium is cytologically malignant

present. The nuclear features vary considerably within the same tumor, ranging from a single layer to stratified nuclei <4 cells in height with loss of the apical mucin vacuole. The range of atypical nuclear features is analogous to those of colorectal adenomatous polyps [6, 18–22]. Mucin granulomas are a result of rupturing of the glands or cysts and should not be misinterpreted as stromal invasion. These granulomas are often localized and do not show the widespread mucin dissection of stroma as in pseudomyxoma ovarii or pseudomyxoma peritonei. The isolated epithelial cells in mucin granulomas are almost always confined to the boundary of the latter (Fig. 10.16) [23].

Intraepithelial Carcinoma (Noninvasive Carcinoma). MBTs with intraepithelial carcinoma refer to tumors without unequivocal stromal invasion but show nuclear stratification of ≥4 cells in height, coarse chromatin, macronucleoli, and a high mitotic rate (Figs. 10.17, 10.18 and 10.19). The cytologic features, rather than the architecture, are more important in determining whether a component of the tumor is intraepithelial carcinoma or not [1, 6, 11, 24, 25]. When these foci are extensive, meticulous sampling for microscopy is necessary to exclude frank stromal invasion.

Microinvasion. IMBTs, particularly in those with intraepithelial carcinoma, may show foci of stromal microinvasion [18, 26]. The definition

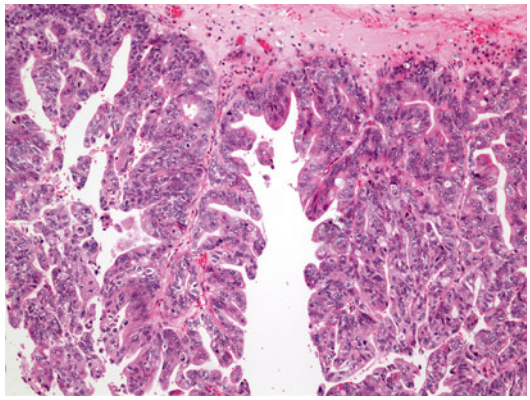

Fig. 10.18 Mucinous borderline tumor, intestinal type, with intraepithelial carcinoma. The malignant glands show back-to-back crowding, but the size of this area is less than that for diagnosing mucinous carcinoma with expansile invasion (see section "Intestinal-type mucinous carcinoma")

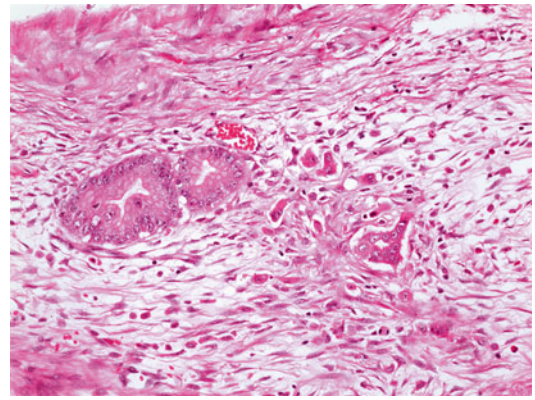

Fig. 10.20 Mucinous borderline tumor, intestinal type, with microinvasion. Invasive tumor cells with eosinophilic cytoplasm and surrounded by a clear space

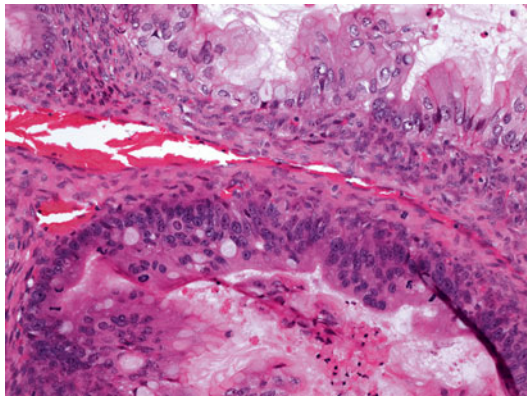

Fig. 10.19 Mucinous borderline tumor, intestinal type, with intraepithelial carcinoma. The intraepithelial carcinoma is usually localized. Note adjacent gland with lesser degree of cytologic atypia

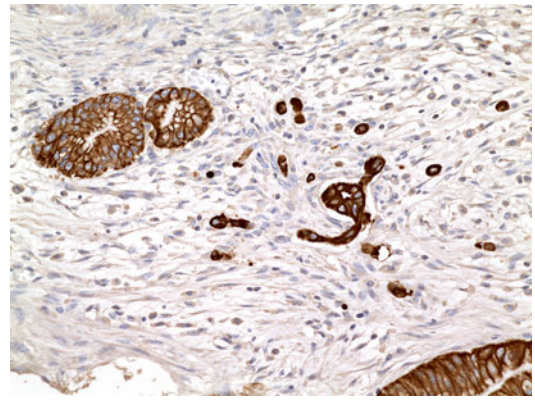

Fig. 10.21 Mucinous borderline tumor, intestinal type, with microinvasion (CK7). Invasive tumor cells may be readily identified using cytokeratin immunostains

of microinvasion varies, but most regard this to be either <5 mm in the greatest dimension or, more specifically, <10 mm^2 (or $\leq 3 \times 3$ mm in two linear dimensions) [24, 25]. Multifocal microinvasion may sometimes be found, but the significance is unknown. The focus usually consists of single cells, solid aggregates, and irregular isolated or confluent glands distributed haphazardly within a reactive fibrous stroma. Cytoplasmic eosinophilia is often seen. The cells are often surrounded by a clear space, which either contains mucin or is due to tissue retraction artifact (Fig. 10.20). The nuclear features in microinvasion are usually mild to moderate but may occasionally be severe. The latter are referred to as "microinvasive carcinoma." Foci of microinvasion may be difficult to appreciate and may go undetected, but a cytokeratin stain may help (Fig. 10.21) [23].

Differential Diagnosis

Mucinous Cystadenoma Versus IMBT: The presence of cribriform glands in cystadenomas, if unaccompanied by cytologic atypia, should not

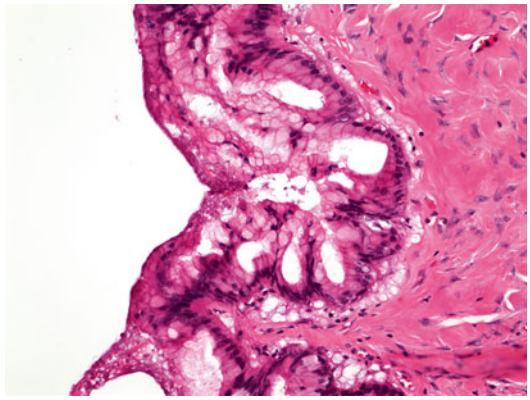

Fig. 10.22 Mucinous cystadenoma. Cribriform glands without cytologic atypia should not be classified as a borderline tumor

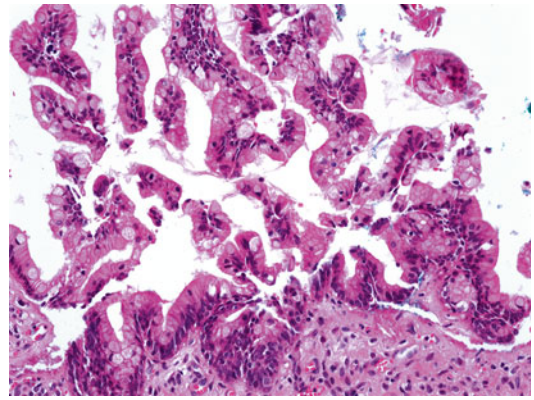

Fig. 10.24 Mucinous cystadenoma with focal atypia. Complex filiform papillae covered by cells with mild-to-moderate atypia

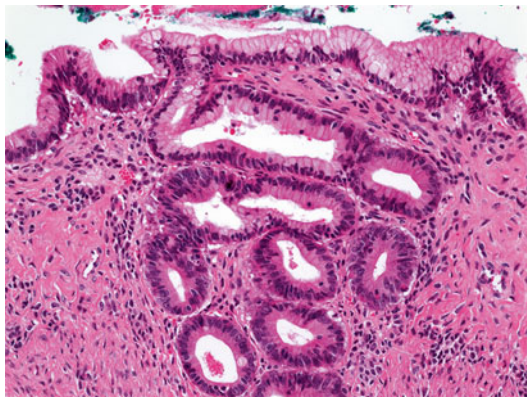

Fig. 10.23 Mucinous cystadenoma with focal atypia. Focal clusters of glands lined by cells with mild-to-moderate atypia. Note benign epithelium over the surface of the cyst lining (*top*)

be classified as borderline tumor (Fig. 10.22). The cribriform arrangement is usually related to tangential sectioning. When there is a minor focus (<10 %) of borderline tumor (i.e., tumor cells with cytologic atypia) in an otherwise benign tumor, the term mucinous cystadenoma with focal atypia (or focal borderline tumor) should be made (Figs. 10.23 and 10.24). The quantity of the borderline tumor (in percentage) should also be stated in the report.

IMBT Versus Mucinous Carcinomas: In mucinous carcinoma, any focus of stromal invasion must exceed that of microinvasion, as defined earlier.

Clinical Outcome and Prognosis

Most stage I IMBTs are benign. In about 6 % of either stage I or stage II/III IMBTs with intraepithelial carcinomas, there is a recurrence [1, 6, 18–22, 26–31]. This is most likely related to under-sampling of a borderline tumor containing occult foci of frankly invasive carcinomas [32]. Stage II/III IMBTs are usually associated with pseudomyxoma peritonei and are in fact almost always secondary tumors to the ovaries, commonly from the gastrointestinal tract (see section "Mucinous tumors associated with pseudomyxoma peritonei") [14, 33]. With rare exceptions, stage I, adequately sampled IMBTs with microinvasion reported had all been clinically benign [1, 6, 18, 21, 26, 31, 34, 35]. However, when the microinvasive tumor cells have high-grade malignant nuclear features, i.e., microinvasive carcinoma, an aggressive clinical behavior may be seen [32]. Treatment by cystectomy alone, as opposed to salpingo-oophorectomy, has been shown to be a significant factor in tumor recurrence [36, 37].

Endocervical-Like Mucinous Borderline Tumor

Synonyms

(Endocervical-like, Müllerian, or seromucinous-type) mucinous borderline tumor; Atypical

Table 10.2 Summary of ovarian mucinous borderline tumors

	Intestinal type	Endocervical-like
Proportion of borderline tumors	85 %	15 % (seromucinous)
Mean age (years)	51	34
Bilaterality	6 %	40 %
Mean size (cm)	19	8
Multilocularity	72 %	20 %
Goblet cells	100 %	0 %
Argyrophil cells	91 %	3 %
Acute inflammation	22 % (focal)	100 % (diffuse)
Endometriosis	6 %	50 %
Pseudomyxoma peritonei	17 %	0 %
Implants or lymph node metastasis	0 %	20 %

Modified and updated from Rutgers and Scully [12]

proliferative mucinous tumor; Mucinous tumor (cystadenoma) of low malignant potential; Mucinous tumor (cystadenoma) of borderline malignancy

Clinical Features

Endocervical-like mucinous borderline tumors (EMBTs) represent 10–15 % of mucinous borderline tumors. In >50 % there is preexisting endometriosis [6, 12, 38, 39]. The age of patients is younger than that for IMBTs, ranging from 19 to 59 years (mean, 34). One-fifth of cases are associated with extraovarian disease at the time of diagnosis. EMBTs have no association with pseudomyxoma peritonei.

Gross Features

EMBTs differ from their intestinal counterpart in several aspects (Table 10.2). They are usually smaller (mean diameter 8 cm), have fewer locules (≤3), are more often bilateral (40 %), have a stronger association with endometriosis (30–50 %), and almost always have an associated inflammatory reaction [12]. Polypoid excrescences, similar to those observed in serous borderline tumors, are usually found (Fig. 10.25) [12].

Microscopic Features

Club-shaped papillae with hierarchical branching, architecturally similar to those seen in serous borderline tumors, characterize EMBTs (Figs. 10.26, 10.27, 10.28, 10.29, 10.30, and 10.31). There is almost always some extracellular mucin, commonly infiltrated by neutrophils. The papillae have edematous fibrovascular cores and are covered by three cell types: mucinous cells, eosinophilic cells, and neutrophils. The mucinous cells are tall columnar and resemble those of the endocervix, while the polygonal eosinophilic cells are similar to those of serous borderline tumors and may show tufting and marked cellular stratification, sometimes to ≥20 cells in height.

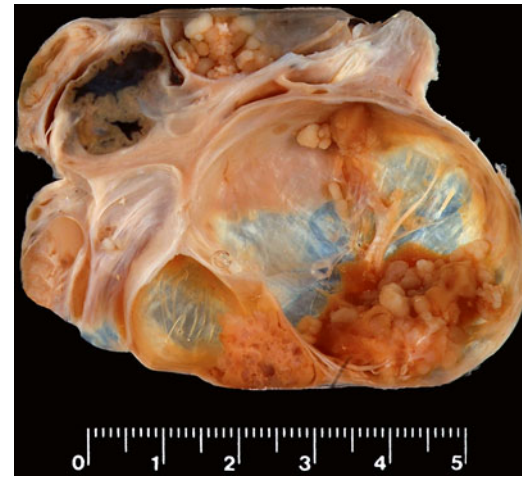

Fig. 10.25 Mucinous borderline tumor, endocervical-like. In contrast to borderline mucinous tumor of intestinal type, this is smaller, has fewer locules, and shows polypoid excrescences over the internal cyst lining

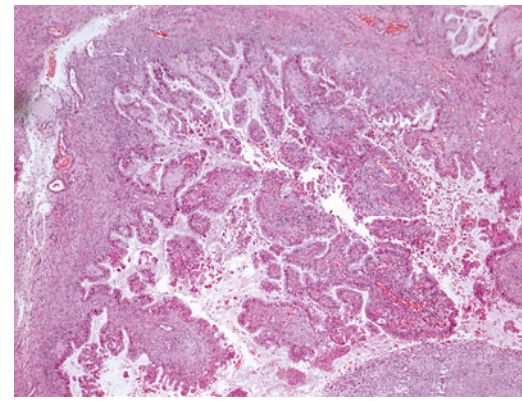

Fig. 10.26 Mucinous borderline tumor, endocervical-like. Club-shaped papillae project into cystic space

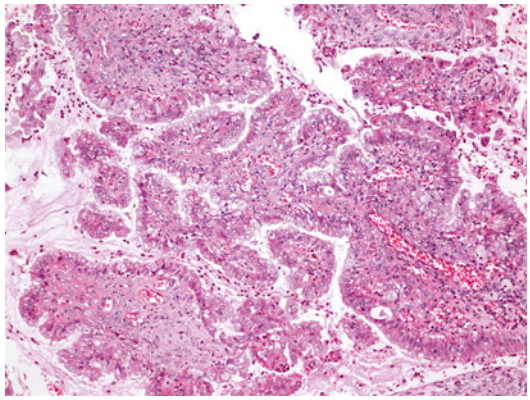

Fig. 10.27 Mucinous borderline tumor, endocervical-like. The papillae show hierarchical branching

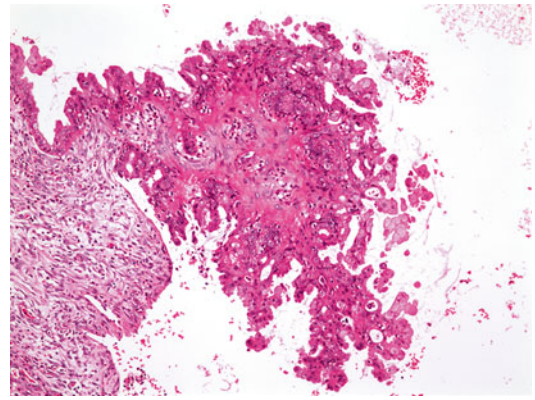

Fig. 10.30 Mucinous borderline tumor, endocervical-like. The cores of papillae are typically infiltrated by numerous neutrophils

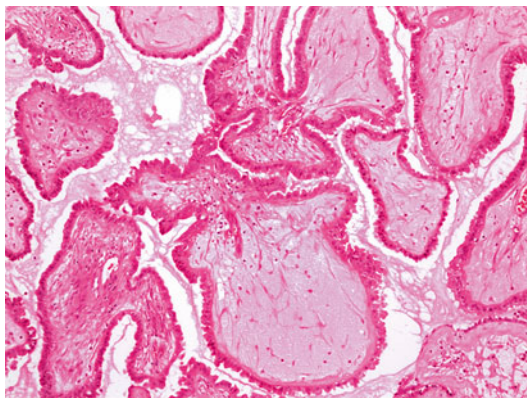

Fig. 10.28 Ovarian serous borderline tumor. The architecture is identical to mucinous borderline tumor, endocervical-like. The epithelial cells are predominantly eosinophilic but occasionally may have mucous cells

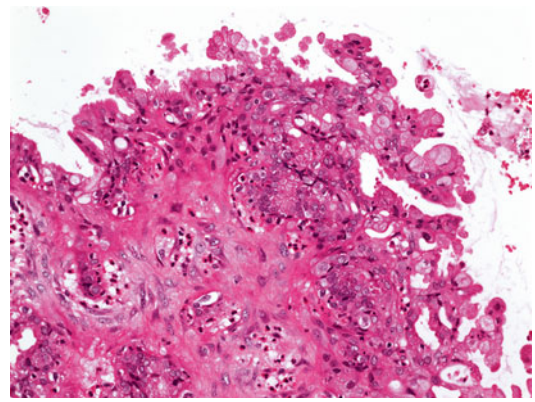

Fig. 10.31 Mucinous borderline tumor, endocervical-like. The epithelial cells may be highly stratified, but they show only mild-to-moderate cytologic atypia

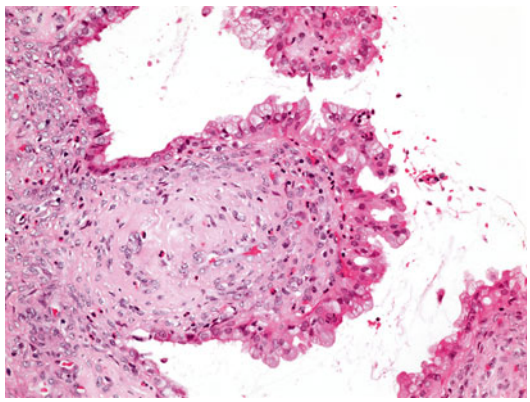

Fig. 10.29 Mucinous borderline tumor, endocervical-like. The papillae are covered by mucous cells and eosinophilic cells

Occasionally, ciliated cells are found. Cytologic atypia is usually mild to moderate although it may be focally severe. Like IMBTs, the combination of cellular stratification and severe atypia in EMBTs may be designated intraepithelial carcinoma (Figs. 10.32 and 10.33). In many cases, endometriosis, including atypical endometriosis, is found in the background cyst lining and may show transition to the EMBT. Microinvasion has been reported in 10–20 % of cases, and the definition is same as for IMBTs (see section "Intestinal-type mucinous borderline tumors"). When the microinvasive tumor cells exhibit only mild-to-moderate atypia, they are regarded as EMBTs with microinvasion; if they are severely atypical, the tumor should be diagnosed as EMBT

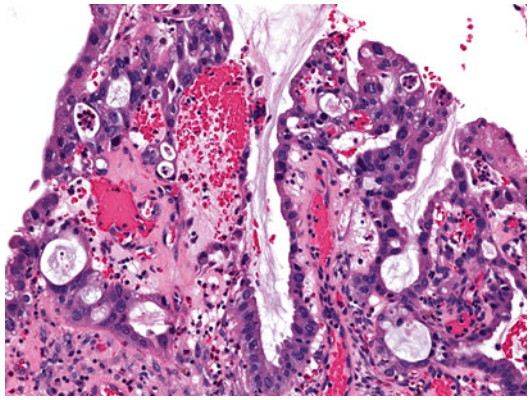

Fig. 10.32 Mucinous borderline tumor, endocervical-like, with intraepithelial carcinoma. There is glandular fusion and early cribriforming

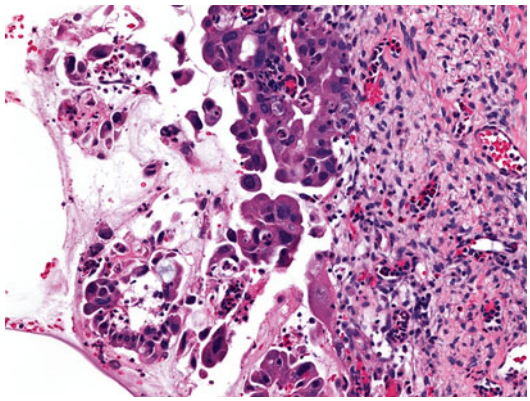

Fig. 10.33 Mucinous borderline tumor, endocervical-like, with intraepithelial carcinoma. The epithelial cells are cytologically malignant. In contrast to Fig. 10.31

with microinvasive carcinoma [34, 35, 40, 41]. The finding of microinvasion or microinvasive carcinoma should prompt additional sampling to exclude frank carcinoma.

Differential Diagnoses

Serous Borderline Tumor Versus EMBT: Serous borderline tumors, which share similar architecture with EMBTs, are distinguished from the latter by the absence of mucinous lining epithelial cells. Neutrophils and extracellular mucin are usually absent or less conspicuous than in EMBTs.

In addition to the mucinous and eosinophilic cells in EMBTs, the presence of serous, endometrioid, or squamous cell types has been reported in these tumors. Even though they were designated as EMBTs of mixed cell types in the older literature [38], they were noted to have the same clinical and pathologic features as EMBTs without these additional cell types, and placing these tumors with mixed cell types into the usual EMBT category is appropriate [41].

Clinical Outcome and Prognosis

The majority of EMBTs are stage I and are almost always followed by a benign clinical course [1, 12, 20, 21, 41, 42]. EMBTs with intraepithelial carcinoma or stromal microinvasion are not associated with a poorer prognosis. Patients with stage II or III disease have peritoneal implants or lymph node metastases, but these were not found to be of any prognostic significance. Fatal cases are exceptional and were described in cases with microinvasive carcinoma or intraepithelial carcinoma, which were most likely due to undetected foci of frankly invasive carcinoma in under-sampled tumors [41, 43].

Mucinous Carcinoma

Intestinal-Type Mucinous Carcinoma

Synonyms

(Intestinal-type) mucinous adenocarcinoma; Cystadenocarcinoma

Clinical Features

Mucinous carcinomas are uncommon and represent 2–7 % of all ovarian carcinomas [44–46]. The higher frequency in earlier studies is most likely related to the inclusion of some metastatic carcinomas that are morphologically indistinguishable from primary ovarian mucinous carcinomas. Patients with primary tumors usually present with an abdominal mass, and the serum levels of CA-125, CEA, and CA19.9 are frequently elevated [47]. The mean age is 45 years. About 75–80 % of cases are stage I. In >95 %, they are unilateral and usually >10 cm (mean, 18–22 cm). When bilateral and each <10 cm, metastases should always be excluded.

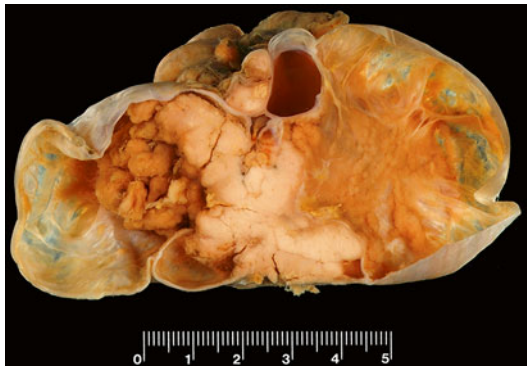

Fig. 10.34 Mucinous carcinoma. The carcinoma may be localized to the solid areas in a seemingly cystic tumor

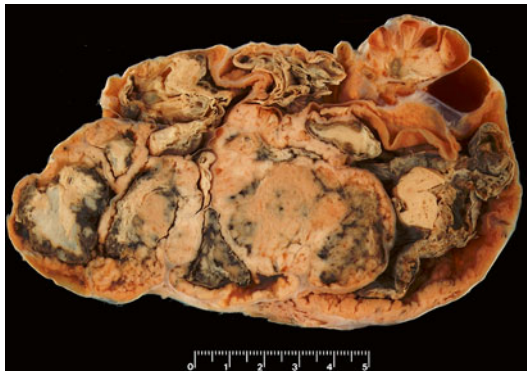

Fig. 10.35 Mucinous carcinoma. A predominantly solid tumor

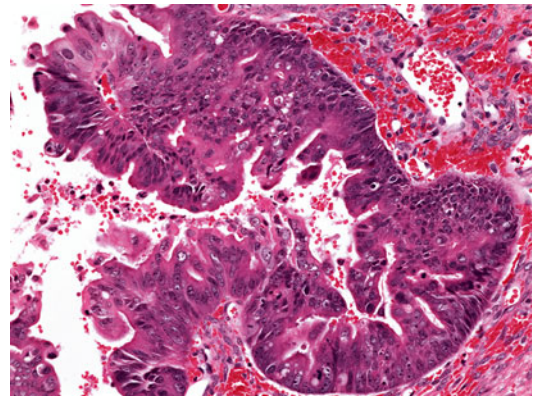

Fig. 10.36 Mucinous carcinoma. The glands are lined by malignant epithelial cells and almost complete loss of cytoplasmic mucin

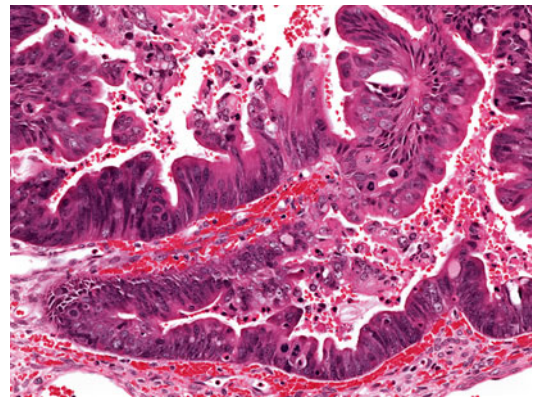

Fig. 10.37 Mucinous carcinoma. The tumor cells show variation in the degree of differentiation. Focally, goblet cells are still discernible

Gross Features

Tumors are usually large, solid, and cystic. Polypoid excrescences may be seen lining the cysts; soft or firm mucoid nodules may be found within the septa of the locules, often accompanied by necrosis and hemorrhage (Figs. 10.34 and 10.35). When sampling for histologic examination, it is crucial to take generous blocks from the solid areas, even though they may only represent a small portion in a large and seemingly cystic tumor.

Microscopic Features

Most cells show intestinal differentiation with prominent goblet cells, but some may represent endocervical-type cells [11]. The epithelium may form complex, crowded, fused, and cribriform glands, complex papillae, or solid nests. The cells usually are stratified to >4 cells in height, with loss of cytoplasmic mucin. The nuclei are severely pleomorphic (Figs. 10.36 and 10.37). In >80 % of carcinomas, there is often a coexisting benign and/or borderline tumor component. Presence of signet ring cells is exceptional, and a metastatic adenocarcinoma, particularly from the stomach, should always be excluded [48].

Mucinous Carcinoma with Expansile Invasion. About 50 % of mucinous carcinomas show expansile-type stromal invasion ("noninvasive," "intraglandular," or "confluent glandular" pattern) [6, 18, 31]. There is florid glandular proliferation without stromal support. The back-to-back

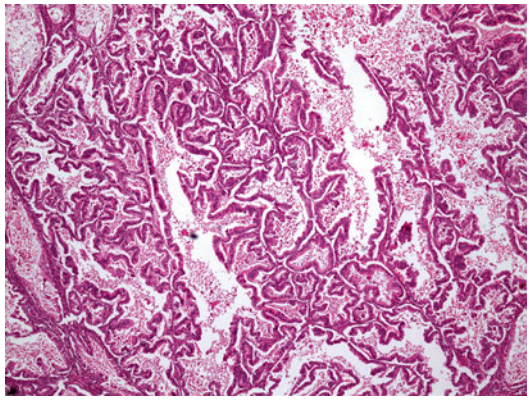

Fig. 10.38 Mucinous carcinoma, expansile invasion. Closely packed glands without intervening stroma

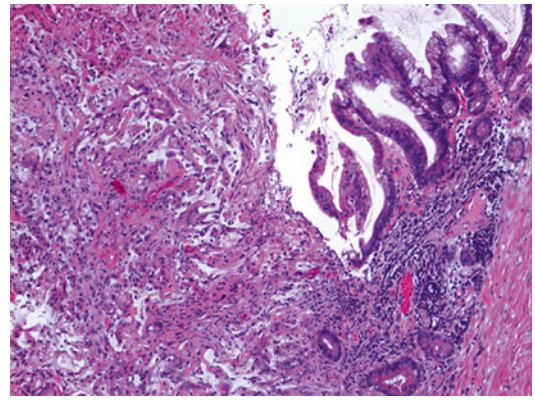

Fig. 10.40 Mucinous carcinoma, infiltrative invasion. Tumor glands irregularly infiltrate into the stroma

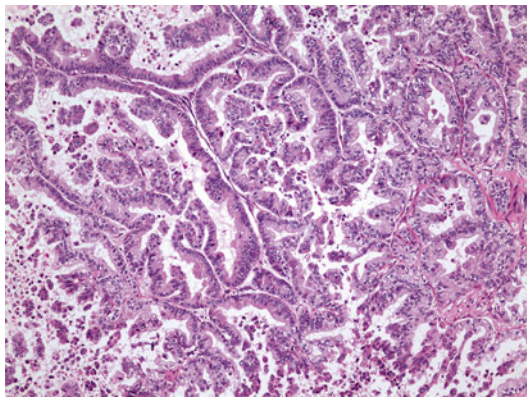

Fig. 10.39 Mucinous carcinoma, expansile invasion

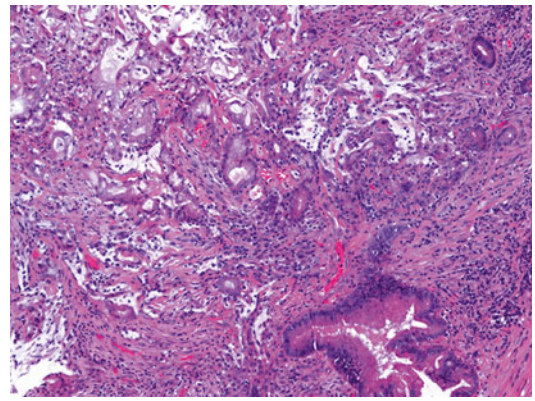

Fig. 10.41 Mucinous carcinoma, infiltrative invasion

cramped glands or cysts have almost no intervening stroma, creating a labyrinth or large cribriform gland patterns (Fig. 10.38 and 10.39).

Mucinous Carcinoma with Infiltrative Invasion. The invasion of interglandular space by irregular glands, cell clusters, or single cells results in destruction of the stroma and/or a desmoplastic reaction (Figs. 10.40, 10.41 and 10.42).

Differential Diagnosis

Mucinous Carcinoma with Expansile Invasion Versus IMBT with Intraepithelial Carcinoma: Distinguishing expansile invasion from IMBTs with intraepithelial carcinoma may be difficult and subjective. To qualify as frank mucinous

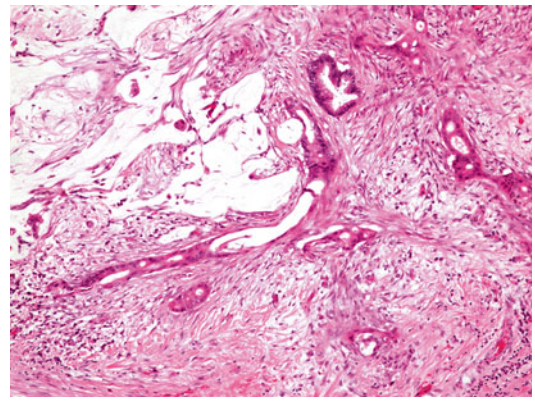

Fig. 10.42 Mucinous carcinoma, infiltrative invasion. Desmoplastic stromal reaction and mucin dissection of stroma

carcinoma, the focus of confluent glands in question should exceed the dimension for microinvasion (see section "Intestinal-type mucinous borderline tumors").

Mucinous Carcinoma Versus IMBT with Microinvasion: The invasive focus should exceed the dimension for microinvasion (see section "Intestinal-type mucinous borderline tumors").

Primary Versus Metastatic Mucinous Carcinoma: Metastatic mucinous carcinomas are more often bilateral, with a mean diameter of <10 cm, and commonly multinodular and show surface deposits. Histologically, a metastasis may show variation in the degree of cytologic atypia, ranging from areas resembling benign or borderline mucinous tumors to those with frankly malignant features. This has been described as the so-called maturation phenomenon and can potentially mimic a primary tumor [49]. The tumor glands are often arranged in nodules with variation in growth pattern between them, with infiltration of normal ovarian follicles or corpus albicans. Vascular invasion is usually prominent [50]. Immunohistochemistry is usually useful (see section "Immunohistochemistry of mucinous tumors").

Endometrioid Carcinoma Versus Mucinous Carcinoma: Endometrioid carcinomas may show abundant luminal mucin or mucin confined to the glycocalyx of the luminal aspect of the tumor cells. These should not be misinterpreted as mucinous carcinomas. Genuine mucinous differentiation should consist of cells with cytoplasmic mucin vacuoles stainable by mucicarmine in >50 % of cells.

Sertoli-Leydig Cell Tumor with Heterologous Elements Versus Ovarian Mucinous Tumor: Rarely, mucinous differentiation in a Sertoli-Leydig cell tumor may be overwhelming, and the gross appearance is that of a mucinous tumor. Histologically, however, typical sex cord tumor components are usually evident (Fig. 10.43) [51].

Prognostic Factors

FIGO stage is most important (Table 10.3). Approximately half of mucinous carcinomas are stage I, and the 5-year survival is 83 %. Those of stages II, III, and IV are 55, 21, and

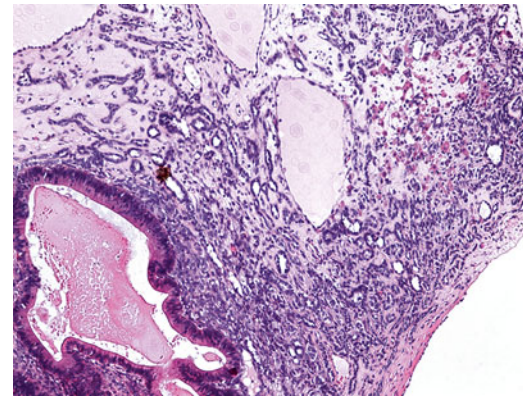

Fig. 10.43 Sertoli-Leydig cell tumor with heterologous mucinous epithelium

Table 10.3 International Federation of Gynecology and Obstetrics Staging System for Ovarian Cancer

I	Tumor limited to ovaries	
	Ia	Tumor limited to one ovary; capsule intact, no tumor on ovarian surface, no malignant cells in ascites or peritoneal washings
	Ib	Tumor limited to both ovaries; capsules intact, no tumor on ovarian surface, no malignant cells in ascites or peritoneal washings
	Ic	Tumor limited to one or both ovaries with any of the following: capsule ruptured, tumor on ovarian surface, malignant cells in ascites or peritoneal washings
II	Tumor involves one or both ovaries with pelvic extension	
	IIa	Extension and/or implants on uterus and/or tube(s); no malignant cells in ascites or peritoneal washings
	IIb	Extension to other pelvic tissues; no malignant cells in ascites or peritoneal washings
	IIc	IIa or IIb with malignant cells in ascites or peritoneal washings
III	Tumor involves one or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis and/or regional lymph node metastasis	
	IIIa	Microscopic peritoneal metastasis beyond the pelvis
	IIIb	Macroscopic peritoneal metastasis beyond the pelvis 2 cm or less in greatest dimension
	IIIc	Peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension and/or regional lymph node metastasis
	IV	Distant metastasis (excludes peritoneal metastasis)

Note: Liver capsule metastasis is stage III; liver parenchymal metastasis is stage IV. Pleural effusion must have positive cytology for stage IV

9 %, respectively [52]. The pattern of invasion appears to be more important than histologic grading. Mucinous carcinomas with expansile invasion are almost always stage I and are followed by an uneventful clinical course [1, 6]. Only two reported cases were associated with aggressive behavior [32]. The majority of stage II–IV mucinous carcinomas and stage I tumors that recurred were those with infiltrative invasion. Most of the latter were stage Ic. In excess of 90 % of patients, with high-stage carcinoma showing infiltrative invasion, died of their disease [6, 21, 22, 26–29]. An architectural grading using the same criteria as the FIGO grading for endometrial adenocarcinoma has not been shown to be useful in predicting outcome in stage I carcinomas [18, 21, 31]. However, higher architectural grade tumors were more likely to present at a higher stage [6]. In our experience, the majority of mucinous carcinomas are architecturally grade 2. In another study, nuclear grade 3 has been shown to be prognostically significant independent of stage [1].

Endocervical-Like Mucinous Carcinoma

Synonyms

(Endocervical-like, Müllerian, or seromucinous-type) mucinous carcinoma

Clinical Features

These are rare compared to those of intestinal type [39, 41]. The mean age of patients is 45 years, and the presentation is similar to those with mucinous carcinoma of intestinal type. There is also a strong association with endometriosis or endosalpingiosis [41]. Bilaterality is found in over 50 % of cases.

Gross Features

The tumors are usually cystic with either one or more locules. Their mean diameter is 12 cm. Polypoid excrescences may be found in the cyst lining, the ovarian surface, or both. The cyst content includes viscous brown to yellow mucin (Fig. 10.44).

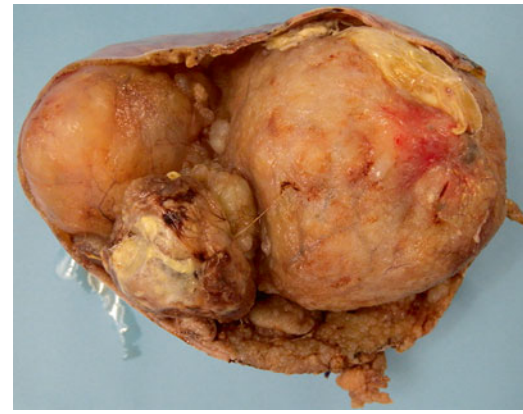

Fig. 10.44 Mucinous carcinoma, endocervical-like. There are often fewer locules compared with intestinal-type carcinomas. Note presence of smaller, polypoid excrescences (lower)

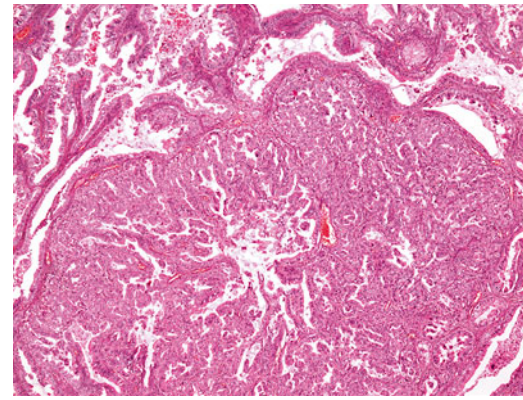

Fig. 10.45 Mucinous carcinoma, endocervical-like, with expansile invasion

Microscopic Features

Almost all endocervical-like mucinous carcinomas are found in association with EMBT. Patients who died of carcinomas of this subtype had tumors that showed, in addition to microscopic features of EMBTs, either frank stromal invasion or only a micropapillary architecture. All of these foci were >5 mm (Figs. 10.45 and 10.46) [41]. In addition to the usual cells types as found in EMBTs, some of these carcinomas also showed varying degrees of serous or endometrioid differentiation.

Prognostic Factors

Prognostic information on endocervical-like mucinous carcinoma is limited. Patients with

stage I tumors were followed by an uneventful outcome. At least half of those with stage II/III disease also had peritoneal invasive implants, two of which had a fatal outcome [39, 41, 53].

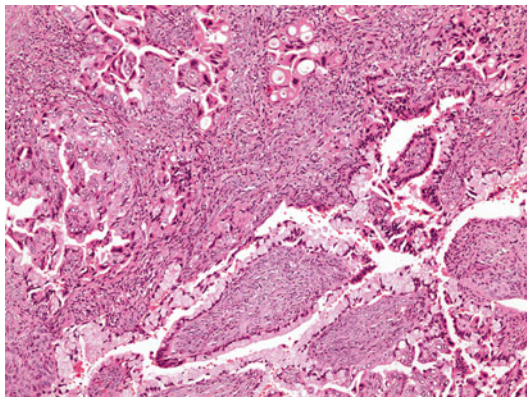

Fig. 10.46 Mucinous carcinoma, endocervical-like, with infiltrative invasion

Immunohistochemistry of Mucinous Tumors

Intestinal-type ovarian mucinous tumors express CK7 (diffusely), CK20, and CDX2 (patchily) (Fig. 10.47) [54–59]. Patchy staining with p16 may be observed and may be useful in distinguishing from metastatic endocervical carcinoma (diffusely positive) [60, 61]. DPC4 is usually expressed in primary mucinous tumors and may be useful for distinguishing from metastatic pancreatic and biliary tract carcinomas (negative in 50 %) [57, 62–64]. Intestinal-type ovarian mucinous tumors are generally nonimmunoreactive for estrogen and progesterone receptors, WT1, and CA-125 [54, 55, 65]. Tumors of teratomatous origin may exhibit typical colorectal carcinoma immunophenotype (CK20+, CDX2+, CEA+, and CK7−), and their use for distinguishing between the two may be impossible [65]. Endocervical-like mucinous

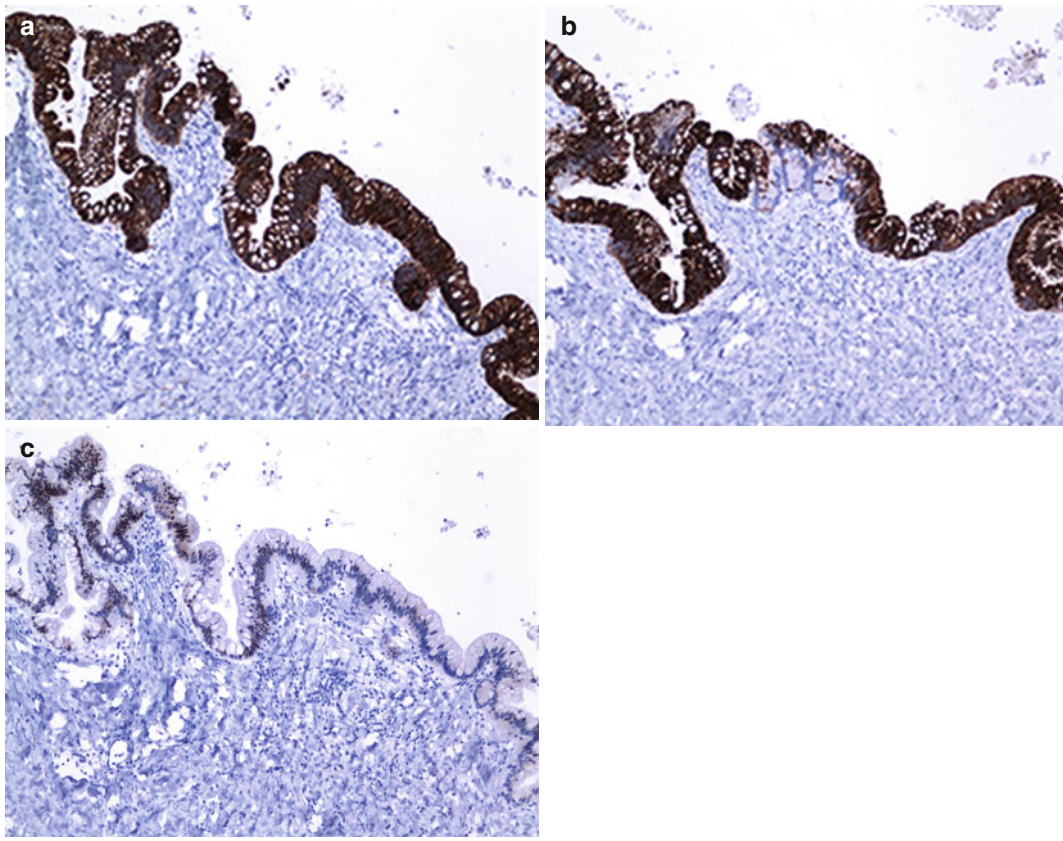

Fig. 10.47 Immunohistochemistry for a typical ovarian mucinous tumor with intestinal differentiation. (**a**) CK7, intense staining in all cells. (**b**) CK20, intense staining in most cells. (**c**) CDX2, moderate staining in a minority of cells

tumors are commonly positive for estrogen and progesterone receptors, CK7, and CA-125, but not for CK20, CDX2, or WT1 [56, 58, 59]. PAX8, a transcription factor for the paired box gene family, has been shown to be expressed in a variety of tumors of Müllerian and mesonephric origin and, in particular, is negative in gastrointestinal tumors and is therefore helpful in the differential diagnosis from metastatic adenocarcinoma. Probably because a large number of mucinous tumors show gastrointestinal differentiation, the expression of PAX8 is usually negative in those cases. In several studies, only 10–40 % of ovarian mucinous carcinomas and 19–23 % of mucinous cystadenomas/borderline tumors were positive for this marker [66–68]. PAX2, a transcription factor related to PAX8, is significantly less specific for use in female genital tract tumors than PAX8. In one study, none of the mucinous carcinomas were positive, and only 19 % of cystadenomas/borderline tumors were positive [66].

Mucinous Tumors Associated with Pseudomyxoma Peritonei

Pseudomyxoma peritonei (PP), or mucinous carcinoma peritonei [69], is a clinical syndrome referring to presence of abundant gelatinous, mucoid material adherent to peritoneal surfaces in the abdomen or pelvis (Fig. 10.48) [70]. Tumor cells are found within the mucin but the cellularity may be very low. The majority of PPs are a result of an appendiceal mucinous neoplasm with ovarian secondaries [14, 71–75]. Rarely, PP may be secondary to an intestinal tumor arising from an ovarian teratoma, commonly in a form of an IMBT [76, 77]. PP can also arise from mucinous carcinomas of other gastrointestinal sites, the breast and even lung, but these are exceptional [69].

In PP, the ovarian tumors are usually bilateral, but may be a unilateral right-sided tumor, often with surface involvement. The histologic appearance resembles ovarian IMBT (Figs. 10.49, 10.50, 10.51, and 10.52). In cases of appendiceal primaries, even though the appendix may be grossly normal, surgical resection with processing in its entirety for histologic examination is necessary.

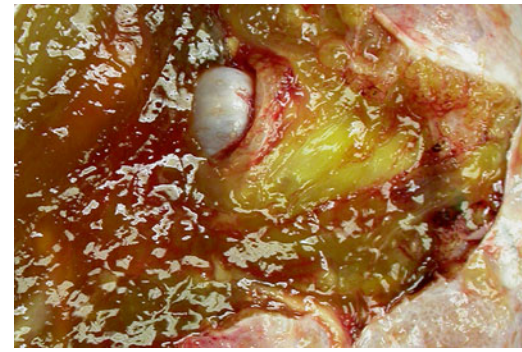

Fig. 10.48 Pseudomyxoma peritonei. Mucoid material occupies the peritoneum

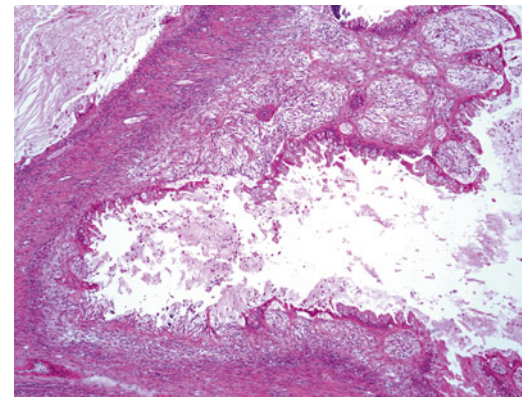

Fig. 10.49 Pseudomyxoma peritonei. Florid mucin granuloma formation in ovaries

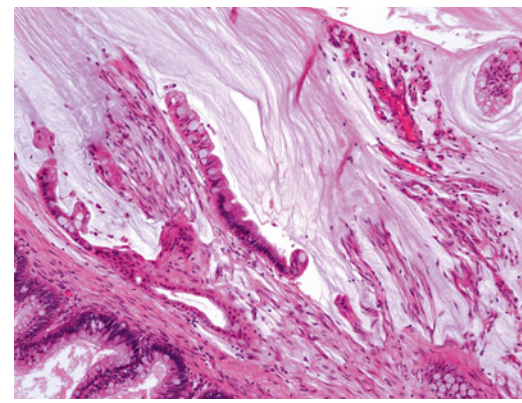

Fig. 10.50 Pseudomyxoma peritonei. Deceptively bland epithelial cells line the glands in the ovaries

This is because the lesion may be small and focal and may or may not show transmural invasion (Figs. 10.53, 10.54, and 10.55). The latter may

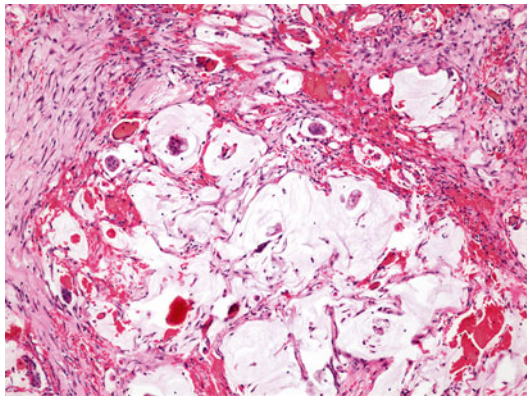

Fig. 10.51 Pseudomyxoma peritonei. Pseudomyxoma ovarii is present

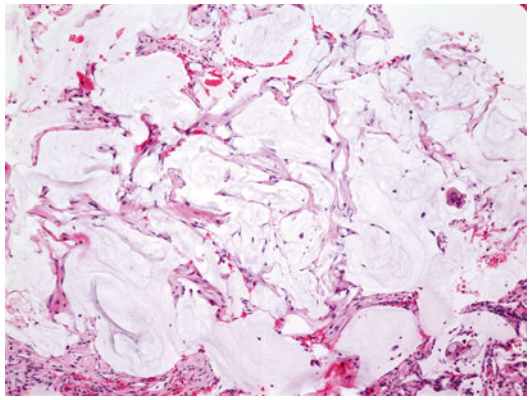

Fig. 10.52 Pseudomyxoma peritonei. Peritoneal mucin may be very low in cellularity

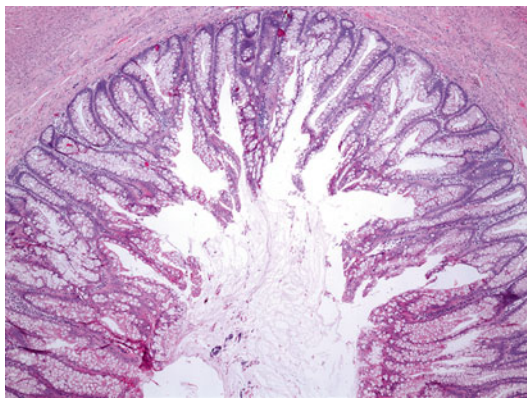

Fig. 10.53 Low-grade appendiceal mucinous neoplasm associated with pseudomyxoma peritonei. Mucosal proliferation without any invasion into the underlying muscularis propria

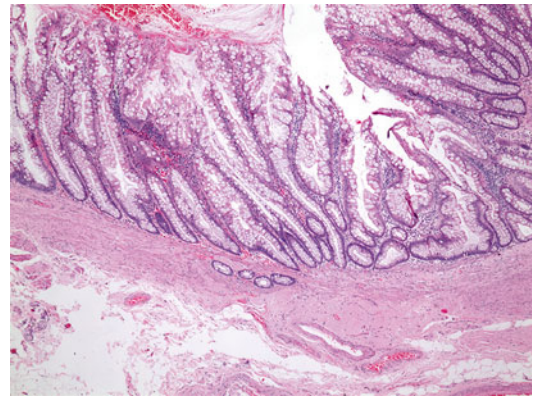

Fig. 10.54 Low-grade appendiceal mucinous neoplasm associated with pseudomyxoma peritonei. The mucosa shows villiform proliferation and covered by deceptively bland epithelium. Note localized thinning of the muscularis propria

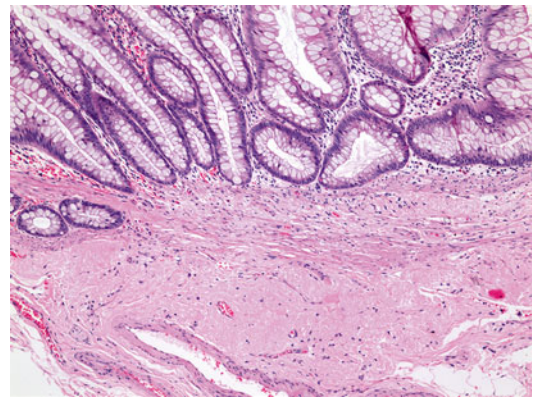

Fig. 10.55 Low-grade appendiceal mucinous neoplasm associated with pseudomyxoma peritonei. Thinning and interruption of the muscularis propria by fibrosis may be the only clue to a previously ruptured site

be due to the fact that the previously ruptured site has been sealed by fibrosis. The tumor may be a low-grade appendiceal mucinous neoplasm or a frank mucinous adenocarcinoma [69]. The incidence of detecting appendiceal mucinous neoplasms in cases of PP with ovarian mucinous tumors is, however, apparently less common in the Asian population [78].

Although immunohistochemistry is helpful in establishing the primary site in most cases, the intestinal-type immunoprofile of the appendix is identical to a minority of IMBTs arising from an

ovarian teratoma. In these cases, thorough histologic examination of the appendix is crucial. Currently, PP should be classified as high or low grade based on the architectural and cytologic features of the tumor cells inside the peritoneal mucin [69].

Mucinous Tumors with Mural Nodules

Benign, borderline, or malignant ovarian mucinous tumors may contain one or more mural nodules which are morphologically different from the preexisting mucinous epithelium. They have been classified into sarcoma-like mural nodules (SLMN), nodules of anaplastic carcinomas (NAC), and true sarcomas. Mixed forms also occur and sometimes it may be difficult to distinguish one from another [79–83]. Despite the favorable prognosis reported in SLMNs and NACs, it should be noted that the prognosis of such lesions would also be dependent on whether or not the preexisting mucinous tumor is a carcinoma and, if so, whether it shows expansile or infiltrative invasion.

SLMNs usually occur in young women (mean, 39 years). Grossly, they represent ≥1 discrete red-brown nodule usually 0.6–6 cm in size and sharply demarcated from the adjacent mucinous tumor. Histologically, the nodule shows a circumscribed proliferation of highly atypical cells comprising multinucleated epulis-like giant cells and malignant-looking spindle cells in a background of inflammatory cells (Figs. 10.56, 10.57, 10.58, and 10.59). The giant cells are usually immunoreactive for histiocytic markers, while the spindle cells are usually negative for cytokeratins [84]. In 50 %, the preexisting mucinous tumor is a carcinoma. Similar cases have been reported in extraovarian sites and rarely have heterologous elements such as osteoid [85]. Of the reported cases of SLMNs, the clinical courses have been benign [82].

NACs may be subclassified according to cell types. These include rhabdoid, sarcomatoid, or pleomorphic. Rhabdoid cells are large and have abundant eosinophilic cytoplasm, eccentric nuclei

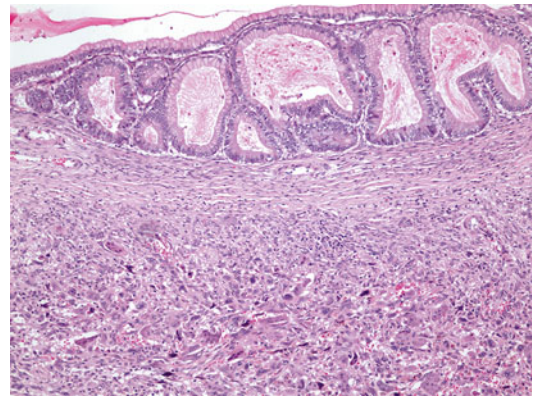

Fig. 10.56 Sarcoma-like mural nodule in mucinous neoplasm. The two components are sharply demarcated from one another

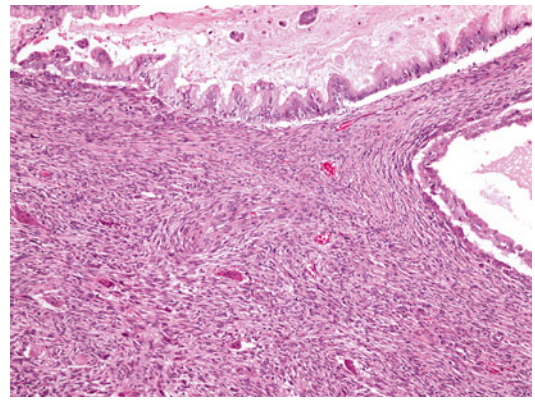

Fig. 10.57 Sarcoma-like mural nodule in mucinous neoplasm. The sarcomatous component contains spindle cells

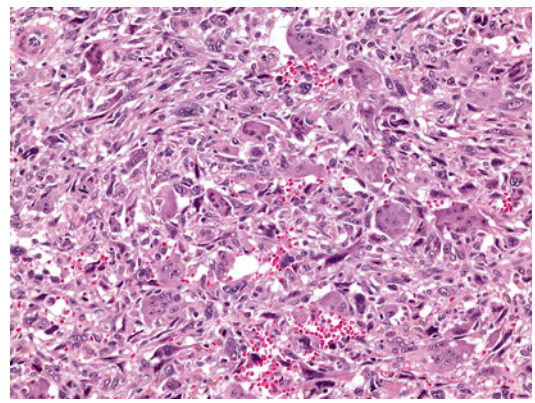

Fig. 10.58 Sarcoma-like mural nodule in mucinous neoplasm. The sarcomatous component is cytologically malignant

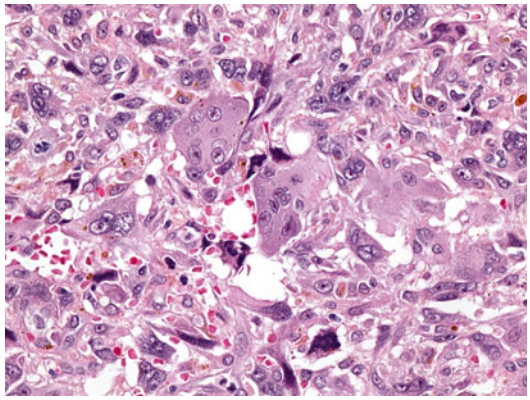

Fig. 10.59 Sarcoma-like mural nodule in mucinous neoplasm. Osteoclast-like giant cells

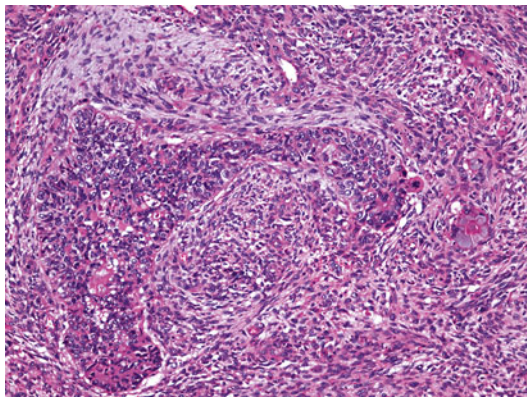

Fig. 10.60 Malignant Müllerian mixed tumor. The carcinomatous and sarcomatous components are well merged together, in contrast to sarcoma-like mural nodules in Fig. 10.56

with prominent nucleoli. The sarcomatoid cells are spindle cells arranged in a herringbone pattern and are immunoreactive for cytokeratin, in contrast to the spindle cells in SLMNs, as noted above. The pleomorphic subtype contains an admixture of rhabdoid and sarcomatoid spindle cells [83]. These nodules may measure up to 10 cm in size and can be multiple. In contrast to SLMNs, NACs have infiltrative borders and show invasion of the surrounding stroma, often with vascular invasion and coagulative tumor necrosis. Although the presence of NAC has not been shown to be an adverse prognostic factor for stage I mucinous tumors, experience with these cases is still limited and distinction from true sarcoma may be difficult in some cases.

True sarcomatous nodules such as fibrosarcoma and rhabdomyosarcoma are aggressive tumors [86, 87].

Mural nodules must be distinguished from carcinosarcoma (malignant mesodermal mixed tumors). In contrast to mural nodules, carcinosarcomas are biphasic and the carcinoma component is intimately mixed or merged with the malignant spindle cells (Fig. 10.60).

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Endometrioid Ovarian Carcinomas 11

Lynn Hirschowitz

Abstract

Endometrioid ovarian tumors include benign, borderline, and malignant categories. Benign and borderline subtypes are uncommon, and endometrioid adenocarcinomas form the vast majority of tumors in this group. As a result of misclassification of ovarian tumors, the precise incidence of endometrioid adenocarcinomas as a percentage of all ovarian carcinomas is difficult to ascertain. In some studies these make up approximately 10 % of all ovarian carcinomas, but in one report they comprise up to 25 %. When very strict criteria are used for their diagnosis, the figure is estimated to be about 7.5 %. In 2009 in the United Kingdom, 6 % of all ovarian surface epithelial-stromal tumors were of endometrioid type (Trent Cancer Registry, personal communication 2012), and according to SEER data (http://seer.cancer.gov/csr/1975_2009_pops09/browse_csr.php?section=21&page=sect_21_table.16.html), 9.7 % of all ovarian surface epithelial tumors in the United States between 2005 and 2009 were of endometrioid subtype.

Introduction

Endometrioid ovarian tumors include benign, borderline, and malignant categories. Benign and borderline subtypes are uncommon, and endometrioid adenocarcinomas form the vast majority of tumors in this group. As a result of misclassification of ovarian tumors [1], the precise incidence of endometrioid adenocarcinomas as a percentage of all ovarian carcinomas is difficult to ascertain. In some studies these make up approximately 10 % of all ovarian carcinomas [2], but in one report they comprise up to 25 % [3]. When very strict criteria are used for their diagnosis, the figure is estimated to be about 7.5 % [4]. In 2009 in the United Kingdom, 6 % of all ovarian surface epithelial-stromal tumors were of endometrioid type (Trent Cancer Registry, personal communication), and according to SEER data (http://seer.cancer.gov/csr/1975_2009_pops09/browse_csr.

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php?section=21&page=sect_21_table.16.html), 9.7 % of all ovarian surface epithelial tumors in the United States between 2005 and 2009 were of endometrioid subtype.

In North America and Europe, endometrioid ovarian adenocarcinoma is the second commonest subtype of ovarian carcinoma and is usually of low FIGO stage (stage I or II) at presentation [2]. However, the dualistic model of ovarian carcinogenesis (see Chap. 8), which subdivides epithelial ovarian cancer into two broad categories based on behavior and genetic profile (types I and II), includes this variant of adenocarcinoma in both categories [5]. Low-grade endometrioid tumors are included with type I tumors, which are generally indolent, low stage at presentation, relatively stable genetically, rarely harbor *TP53* mutations, and characterized by specific mutations which target particular cell signaling pathways. High-grade endometrioid tumors on the other hand are classified with type II tumors, which are aggressive, present at an advanced stage, genetically unstable, and have a high incidence of *TP53* mutations. High-grade endometrioid tumors constituted only 11.3 % of high-grade ovarian carcinomas in one study and 2.5 % of all stage III/IV ovarian carcinomas [6].

Kurman and Shih [7] suggested that because of the recognized association of endometriosis with both endometrioid and clear cell ovarian adenocarcinomas [8], endometriosis should be regarded as the precursor of these tumors (see Chap. 2). The authors stated that since it is generally accepted that retrograde menstruation gives rise to endometriosis [9], it is reasonable to assume that the endometrium is the source of these ovarian neoplasms which develop from endometrial tissue implanted onto the surface of the ovary. Epidemiological evidence that shows a protective effect of tubal ligation for ovarian endometrioid and clear cell adenocarcinoma only lends support to this theory [10], although the authors ascribed the association to "a hormonal mechanism." It is recognized that hormonal influences also have a role in the neoplastic transformation of any implanted endometrial tissue. A recent large, Danish cohort study found an increased risk of developing serous and endometrioid ovarian adenocarcinomas in postmenopausal women who used unopposed oral estrogen therapy or estrogen/progestin therapy [11]. Wei et al. [12] described the stepwise progression from endometriosis, atypical endometriosis, and ovarian borderline tumor to endometriosis-associated endometrioid ovarian carcinoma. The authors also reviewed the role of steroid hormones, inflammation, and genetic alterations on the process of neoplastic transformation. A recent study identified a new isoform of steroid receptor coactivator1 that has a critical role in promoting neoplastic transformation of endometriosis in mice [13].

Benign Endometrioid Ovarian Tumors

Clinical Associations and General Features

Endometrioid adenofibromas and cystadenofibromas are uncommon tumors, usually occur in association with borderline endometrioid tumors, and are associated with endometriosis [8]. These tumors are usually unilateral and occur at a median age of 57 years [4].

Macroscopic Features

These tumors are of mixed solid-cystic type and have a smooth external surface and a solid, fibrous, cut surface with small, interspersed cysts that impart a spongelike appearance. The cysts contain clear or straw-colored fluid, and there may be small papillary structures at the luminal surface of the cysts.

Microscopic Features

The stroma is usually fibromatous and surrounds architecturally simple, endometrioid epithelial elements. These comprise tubular, branching, or small cystic glands (Fig. 11.1). The glands resemble inactive eutopic endometrial glands but

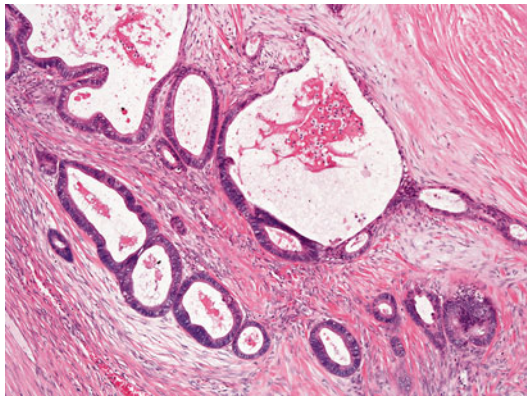

Fig. 11.1 Benign endometrioid adenofibroma. These tumors contain simple, tubular endometrioid glands that are separated by hyaline collagenous stroma

may show proliferative features or even changes resembling simple, non-atypical endometrial glandular hyperplasia. The lining epithelium is of tall columnar, endometrioid type, with bland, oval nuclei that have coarse nuclear chromatin and small nucleoli. Mitotic activity is low but can be prominent, which imparts a resemblance to proliferative phase endometrium. Squamous differentiation is often seen, as are secretory changes and tubal/ciliated cell metaplasia.

Differential Diagnosis

The diagnosis is not usually problematic but it is important to ensure that such tumors are adequately sampled to ensure that borderline areas are not overlooked. The presence of ciliated/tubal epithelium may occasionally raise the possibility of serous cystadenofibroma, but the lack of large (often multiple) cysts and the absence of broad-based papillae with fibrous, focally hyalinized cores favor a diagnosis of endometrioid cystadenofibroma.

Treatment and Behavior

Although these tumors do, rarely, recur, they are benign and are adequately treated by oophorectomy.

Borderline Endometrioid Ovarian Tumors

Clinical Associations and General Features

These tumors are also known as atypically proliferative endometrioid tumors. Although they comprised 2–19 % of ovarian tumors and 2–10 % of all borderline tumors in an older study [14], according to Seidman et al. [4] they comprise only 0.2 % of ovarian epithelial neoplasms. In the United Kingdom in 2009, borderline endometrioid ovarian tumors accounted for approximately 3 % of all borderline ovarian tumors (Trent Cancer Registry, personal communication). These tumors occur in the older reproductive and postmenopausal age groups [15]. In one study of 30 patients with this tumor type, the age range was 28–86 years (mean 54.9 years) [16]. In the same study, 4 % of patients had bilateral tumors, 63 % had endometriosis, and 39 % of the patients from whom an "endometrial specimen" was available had endometrial hyperplasia or carcinoma.

Macroscopic Features

The tumors are usually unilateral, cystic, or solid-cystic with a smooth capsular surface. In one study the tumors ranged from 1 to 22 cm across (mean diameter 10.7 cm) [16]. The cut surface in the solid areas is of variegated gray and light brown appearance and contains small cysts with hemorrhagic or brownish green contents (Fig. 11.2).

Microscopic Features

These tumors characteristically show a range of epithelial proliferative changes that mirror the neoplastic transformation sequence in eutopic endometrium. The proliferating epithelial glands are set in a fibromatous or adenofibromatous background and have a nodular architecture (Fig. 11.3). Underlying adenofibroma was present in just under half of the cases in the

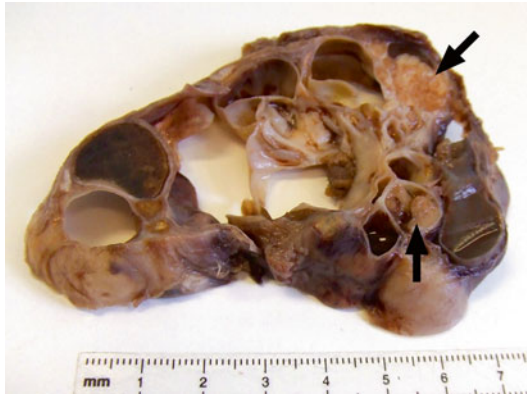

Fig. 11.2 Borderline endometrioid adenofibroma. These tumors are typically part solid and part cystic. The cysts are of variable size and contain gelatinous brown material. The solid components (*arrows*) may project into the lumina of the cysts

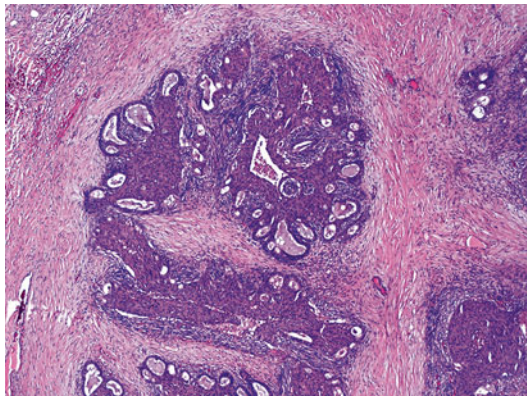

Fig. 11.3 Borderline endometrioid tumor. The proliferating endometrioid glands have a complex architecture and include areas of squamous metaplasia. The proliferating glands are set in a fibromatous stroma and typically have a nodular growth pattern

series reported by Roth et al. [16], and endometriosis was reported in 35–63 % [16, 17]. Borderline endometrioid tumors show a greater degree of epithelial proliferation than adenofibromas, with an increased gland/stroma ratio, architectural complexity (occasionally including the formation of papillary structures), and a more prominent cribriform pattern of growth. The glandular architectural complexity is accompanied by cytological atypia which is usually mild, but may be moderate or severe. Squamous morules are common and may occasionally be extensive and confluent. Necrosis is often seen in gland lumina or cysts.

Confluent glandular epithelial proliferation (so-called stromal disappearance) [16] is considered to indicate stromal invasion, and if this feature or destructive stromal invasion of <5 mm in greatest linear dimension or <10 mm^2 in greatest area is identified on one slide [17], the term microinvasion is applied: i.e., such tumors are designated borderline endometrioid tumors with microinvasion. Confluent epithelial proliferation or frank, destructive stromal invasion >5 mm warrants a diagnosis of carcinoma.

Although the stroma is fibrotic, there may be mild stromal hypercellularity around the glandular elements, but there is no stromal atypia or stromal mitotic activity, and a well-formed cambium layer is absent. These features should allay the suspicion of low-grade adenosarcoma.

Differential Diagnosis

When there is an expansile growth pattern, the main area of diagnostic difficulty lies in differentiating a borderline endometrioid ovarian tumor from endometrioid adenocarcinoma. The criteria are similar to those applied when differentiating complex atypical endometrial hyperplasia from endometrioid adenocarcinoma in the endometrium. The following features are considered to be evidence of invasion: extensive gland confluence with a cribriform growth pattern, a back-to-back glandular architecture, a papillary architecture, or a serpiginous, mazelike growth pattern with stromal loss.

It is uncommon to encounter diagnostic difficulty in differentiating benign from borderline endometrioid tumors. The latter show epithelial proliferation, glandular architectural complexity, and cytological atypia, whereas the former lack these defining features.

Treatment and Behavior

Even when microinvasion and severe cytological atypia are present, these tumors tend to present

at an early stage [15, 16] and have an excellent prognosis [18]. For women of childbearing age, unilateral salpingo-oophorectomy and follow-up are advised, but when retention of fertility is no longer important, bilateral salpingo-oophorectomy is recommended. According to Murray and Park, because of the very low rate of metastasis, a staging procedure is not necessary [17]. There is no need for lymphadenectomy. Underlying endometrial pathology should be excluded because of the reported association of borderline endometrioid tumors with endometrial hyperplasia and carcinoma [16].

Ovarian Endometrioid Adenocarcinoma

Clinical Associations and General Features

Endometrioid ovarian adenocarcinoma occurs mainly in postmenopausal women in the fifth and sixth decades [3], at a median age of 60 years (2 years younger than the median age of 62 years at diagnosis of serous ovarian carcinoma).

Pelvic endometriosis is associated with ovarian endometrioid adenocarcinoma in 26–41.9 % of cases [19–23], and an association with atypical endometriosis is recognized [20]. On average, patients whose tumors develop in association with endometriosis are 5–10 years younger than those who develop tumors in the absence of endometriosis [24]. 10–12.4 % of patients have synchronous ovarian and endometrioid adenocarcinomas [25, 26]. Women with synchronous ovarian and uterine tumors of low histological grade limited to the uterus and ovary have a surprisingly good prognosis [21, 27], with better survival than those patients having only an ovarian tumor [3]. In women aged 40 or younger, rates of synchronous carcinomas are higher, from 11 to 23 % [26, 28]. Whenever simultaneous tumors are diagnosed, it is important to ascertain if the tumors represent independent primary tumors or metastatic disease from one gynecological site to the other. There are well-established criteria to differentiate between these two situations (see Chap. 16).

Most ovarian endometrioid carcinomas, like ovarian carcinomas in general, are asymptomatic. Patients may present with abdominal distension, abdominal or pelvic pain, pelvic mass, abnormal vaginal bleeding if associated with an endometrial neoplasm, or symptoms related to underlying endometriosis if this is present [22]. A pelvic mass is usually identified on clinical examination, and serum levels of CA125 are raised in most cases [29, 30].

Macroscopic Features

Tumors are unilateral in a majority of cases [17] and generally of mixed solid-cystic type, are 10–20 cm in diameter [31], and have a smooth capsular surface unless associated with endometriosis when capsular adhesions and foci of capsular endometriosis may be present (Fig. 11.4a). Slicing reveals cysts with mucoid or greenish/brown contents and soft, friable tumor in the cyst wall or protruding into the cyst lumen (Fig. 11.4b). Occasionally tumors may be solid and show hemorrhage and necrosis. When there is associated endometriosis, "chocolate cysts" with luminal excrescences or solid tumor in the cyst wall may be identified.

Microscopic Features

The morphological spectrum of ovarian endometrioid adenocarcinomas is similar to that of invasive endometrioid adenocarcinomas in the uterus. Well-differentiated, ovarian endometrioid adenocarcinomas account for the majority of tumors. The architecture may be glandular (Fig. 11.5), cribriform, villoglandular, or papillary (Fig. 11.6) and comprises back-to-back, near-confluent glands that have smooth, rounded luminal contours, in contrast to the irregular, slit-like luminal contours of the glands in serous tumors. The gland lumina may contain mucin or eosinophilic material. The glands are lined by stratified, tall columnar epithelial cells with round to oval vesicular nuclei and inconspicuous nucleoli. The scanty cytoplasm is eosinophilic but may be

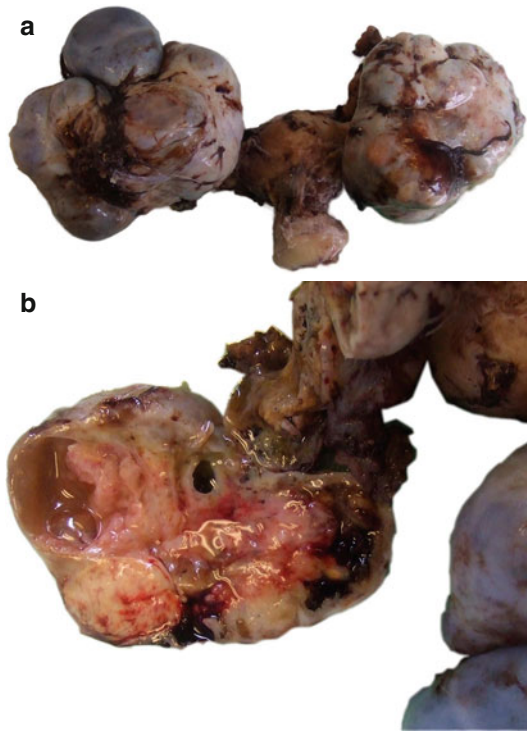

Fig. 11.4 Bilateral ovarian endometrioid adenocarcinomas. (**a**) These ovarian tumors are part solid and part cystic. This example has a hemorrhagic capsular surface as a result of associated endometriosis. (**b**) The cut surface shows soft, nodular tumor tissue which protrudes into the cyst lumen. The tumor contains foci of hemorrhage and necrosis

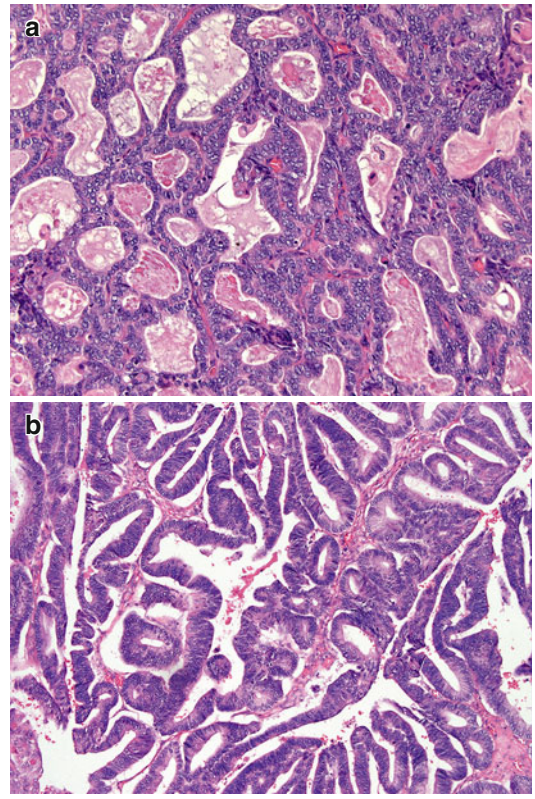

Fig. 11.5 Ovarian endometrioid adenocarcinoma with a glandular architecture. (**a**) The gland lumina often contain eosinophilic material. The columnar endometrioid cells typically show low-grade cytological features. (**b**) This example is composed of architecturally complex, anastomosing endometrioid glands with low-grade cytological features

mucinous focally (Fig. 11.7). Although there is a low-grade architecture, high-grade nuclear features may be identified focally. In general, the cytological features parallel the degree of architectural differentiation, with higher-grade tumors that have a sheetlike, solid growth pattern composed of markedly atypical epithelial cells. Moderate and poorly differentiated endometrioid adenocarcinomas usually show solid, complex glandular or microglandular growth patterns, with marked cytological atypia, nuclear pleomorphism, and increased mitotic activity.

Metaplastic changes are common, and squamous metaplasia (or differentiation) is identified in up to 50 % of cases, usually in the form of morules or balls. The morules are located in areas of gland confluence, usually in the center of the neoplastic glands. The squamous cells appear cytologically benign, and as in the case

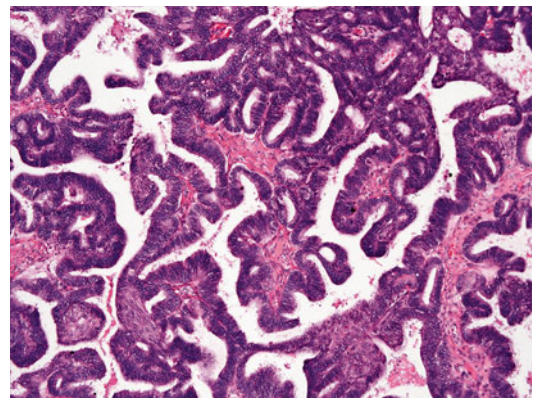

Fig. 11.6 Ovarian endometrioid adenocarcinoma with a papillary architecture and low-grade cytological features

of endometrial adenocarcinoma with morular/squamous metaplasia, ovarian endometrioid tumors should be designated "endometrioid

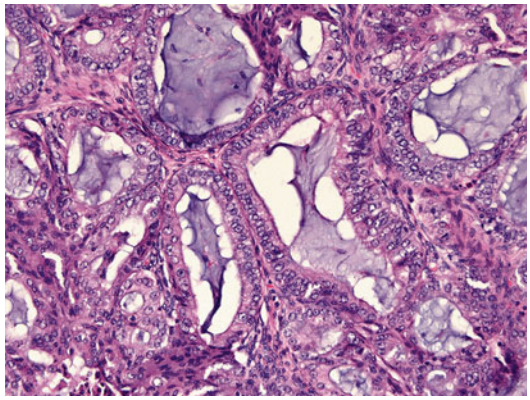

Fig. 11.7 Ovarian endometrioid adenocarcinoma with mucinous differentiation. The mucin-filled acini are surrounded by columnar cells with low-grade features

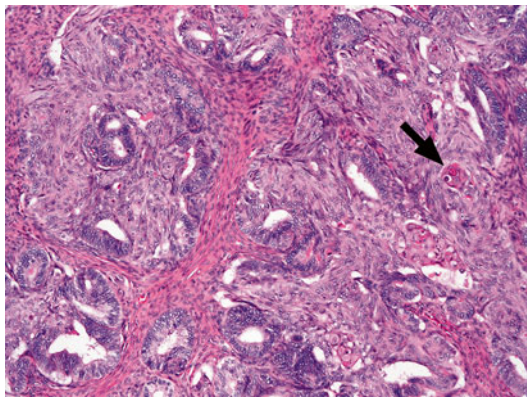

Fig. 11.8 Ovarian endometrioid adenocarcinoma with squamous differentiation/morule formation. This example shows coalescent squamous differentiation with focal keratinization (*arrow*)

adenocarcinoma with squamous differentiation," rather than "adenoacanthoma" or "adenosquamous carcinoma" [31]. Squamous morules may sometimes have a spindled morphology, but the nests usually remain discrete within the coalescing, neoplastic endometrioid glands, and the presence of more typical squamous morules elsewhere helps with their identification. The squamous morules can undergo keratinization (Fig. 11.8) or degenerative changes, which may provoke a foreign-body giant cell reaction in the tumor stroma. Some endometrioid ovarian adenocarcinomas have a more extensive spindled appearance which may cause diagnostic confusion with other spindle-cell neoplasms – this variant of endometrioid adenocarcinoma is discussed below. Other forms of metaplasia that may be seen in endometrioid ovarian adenocarcinoma include secretory, hobnail, ciliated cell, mucinous, and oxyphilic/eosinophilic metaplasia; rarely one of these forms of differentiation predominates. Mucinous differentiation is usually focal; well-differentiated, mucinous ovarian adenocarcinomas should contain >50 % of mucinous cells to warrant designation as such. Secretory change may also result from progesterone administration, particularly in patients who have been treated for vaginal bleeding.

Luteinized stromal cells are present in 12 % of cases [4]. Keratin granulomas in the peritoneal cavity have been reported in patients with ovarian endometrioid carcinoma with squamous differentiation and keratinization [32, 33]. These are likely to result from spillage as a result of tumor rupture. The granulomas must be examined carefully to exclude peritoneal tumor deposits. Follow-up of patients with peritoneal keratin granulomas has shown these to be of no prognostic significance [32, 33].

Endometriosis is often present, and when tumors arise from preexisting benign or borderline endometrioid tumors, it is not unusual to see a spectrum of changes from endometriosis through benign and borderline endometrioid tumors to invasive ovarian endometrioid adenocarcinomas. Diagnosing an invasive component may be problematic in some cases, particularly when preexisting benign or borderline endometrioid tumors are present. There are two recognized patterns of invasion [18], the first of which – destructive stromal invasion – is easier to identify but less often present (Fig. 11.9). This pattern of invasion resembles the typical pattern of myoinvasive endometrioid endometrial adenocarcinoma: irregular, angulated, infiltrative glands with smaller, jagged nests, groups, or single tumor cells extending into desmoplastic stroma. There may also be an edematous or inflammatory stromal reaction. The second pattern of invasion is commoner and is described as "expansile" or "confluent" (Fig. 11.10). In this pattern of invasion, there is confluent growth of

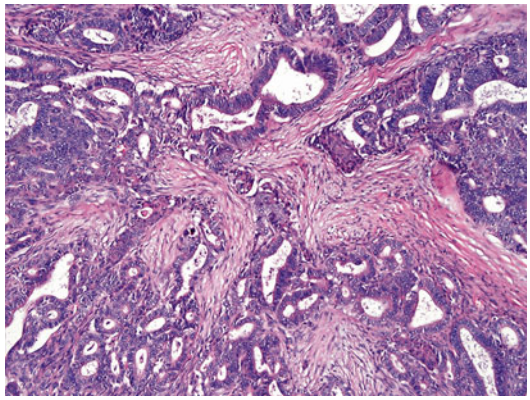

Fig. 11.9 Ovarian endometrioid adenocarcinoma with an infiltrative, destructive pattern of invasion. Jagged nests of irregular endometrioid glands infiltrate desmoplastic stroma

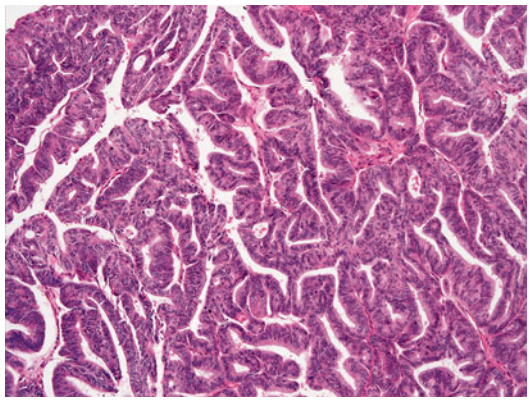

Fig. 11.10 Ovarian endometrioid adenocarcinoma with an expansile or confluent growth pattern, characterized by complex endometrial glandular proliferation without intervening stroma

epithelium which excludes stroma. The confluent epithelial growth can be cribriform, show extensive gland budding or branching, have a mazelike architecture, or show a highly complex, papillary proliferation. It is important to recognize this expansile pattern of invasion to avoid underdiagnosis of adenocarcinoma as a borderline tumor. The expansile pattern of invasion should exceed 5 mm, which is the limit for microinvasion.

Murray and Park [17] suggested that "confirmatory endometrioid features" may be helpful in the diagnosis of endometrioid adenocarcinoma, particularly in cases of poorly differentiated or high-grade endometrioid adenocarcinomas with a solid sheetlike, papillary or micropapillary architecture and focal slit-like glandular spaces. In these circumstances it may be difficult to exclude high-grade serous carcinoma, and in their institutional study, the presence of at least one "confirmatory endometrioid feature" proved to be useful to separate poorly differentiated ovarian tumors into specific, clinically relevant groups. The authors recommended that a diagnosis of endometrioid adenocarcinoma should be avoided when "confirmatory endometrioid features" are lacking unless there is immunohistochemical or genotypic information to support the diagnosis. The "confirmatory endometrioid features" are:

- Morphological similarity to endometrium and to endometrial, endometrioid uterine tumors
  - Architectural features: cribriform or tubular glands with smooth luminal contours; papillae or a sheetlike growth pattern
  - Cytological features: columnar cells; cytological grade in keeping with the degree of architectural differentiation
- Metaplastic changes: squamous/morular, hobnail, eosinophilic, and mucinous metaplasia; secretory change
- Associated features: endometriosis, preexisting benign or borderline endometrioid ovarian neoplasm, or synchronous endometrioid, endometrial adenocarcinoma

Grading (Table 11.1)

There is no widely accepted grading system for ovarian endometrioid adenocarcinoma. The most commonly used system (recommended in the Royal College of Pathologists' Ovarian Cancer Dataset) [34] is that used to grade endometrial endometrioid adenocarcinomas, i.e., the combined architectural and cytological FIGO grading system [35]. Architectural grading should be based only on the percentage of nonsquamous solid growth; the presence of significant cytological atypia in a majority of tumor cells raises a tumor of architectural grade 1 or 2 by one grade. A recent study suggests that

Table 11.1 Grading systems for endometrioid ovarian adenocarcinoma

FIGO grading system for endometrioid ovarian carcinoma			
Architectural grade			
Grade 1	≤5 % non-squamous, solid growth pattern		
Grade 2	6–50 % non-squamous, solid growth pattern		
Grade 3	>50 % non-squamous, solid growth pattern		
Combined FIGO grade: "notable" or significant nuclear atypia raises the overall grade of architectural grade 1 or 2 tumors by one point			
Shimizu/Silverberg grading system for ovarian carcinomas			
	Score 1	*Score 2*	*Score 3*
Predominant architectural pattern	Glandular	Papillary	Solid
Cytological atypia[a]	Slight	Moderate	Marked
Mitotic figures/10 high-power fields	0–9	10–24	≥25
Total score:	3–5	Grade 1 (well differentiated)	
	6 or 7	Grade 2 (moderately differentiated)	
	8 or 9	Grade 3 (poorly differentiated)	

[a]Based on an area of tumor comprising at least 50 % of a low-power (×4 objective) microscopic field with the greatest degree of atypia. Assessment of atypia is based on variation in nuclear size and shape, chromatin texture, nuclear/cytoplasmic ratio, and the prominence of nucleoli

differentiating grade 1 from grade 2 adenocarcinoma is not clinically relevant in endometrial tumors [36], and it is presumed that the same applies to ovarian endometrioid tumors. Another system that has been used is the Shimizu/Silverberg grading system [37–39]. This was found to correlate with prognosis for all histological subtypes of ovarian carcinoma except clear cell carcinoma in some but not all studies [39, 40]. The grading system involves assigning scores from 1 to 3 for architecture, cytological atypia, and mitotic activity and then adding and grouping the scores to give 3 tumor grades in a similar way to the Nottingham grading system for breast carcinoma. The Shimizu/Silverberg grading correlated better than the FIGO grading system with 5-year survival rate and was a better predictor of lymph node metastasis [41].

Variants of Ovarian Endometrioid Adenocarcinoma

Sex Cord-Like or Sertoliform Variant [42]

Unusual microscopic patterns of ovarian endometrioid tumors (which have also been described in their endometrial counterparts [43]) include tumors with morphological features that mimic a range of sex cord-stromal tumors, including granulosa cell, Sertoli cell, and Sertoli-Leydig cell tumors. Most such tumors include foci of conventional endometrioid ovarian carcinoma (including areas with squamous metaplasia or areas with an adenofibromatous background) which blend with the areas that show sex cord-like features.

The presence of solid (Fig. 11.11a) or small, hollow, anastomosing tubules (Fig. 11.11b) that are lined by low columnar or cuboidal epithelial cells and set in a fibromatous, focally luteinized background can mimic a well-differentiated Sertoli-Leydig cell tumor. The lumina of the tubules may contain eosinophilic secretions. Another uncommon pattern of growth is that of large islands of small epithelial cells with round vesicular nuclei and scanty cytoplasm, which resemble granulosa cells (Fig. 11.12). The presence of large, dilated glandular structures that resemble follicle-like spaces may further simulate adult granulosa cell tumor. However, the sheets of epithelial cells in the islands do not contain the typical grooved nuclei of a granulosa cell tumor, the range of architectural patterns of adult granulosa cell tumor is not present, and the immunoprofile is not consistent with that of a sex cord-stromal tumor (See section on Differential Diagnosis below) [44].

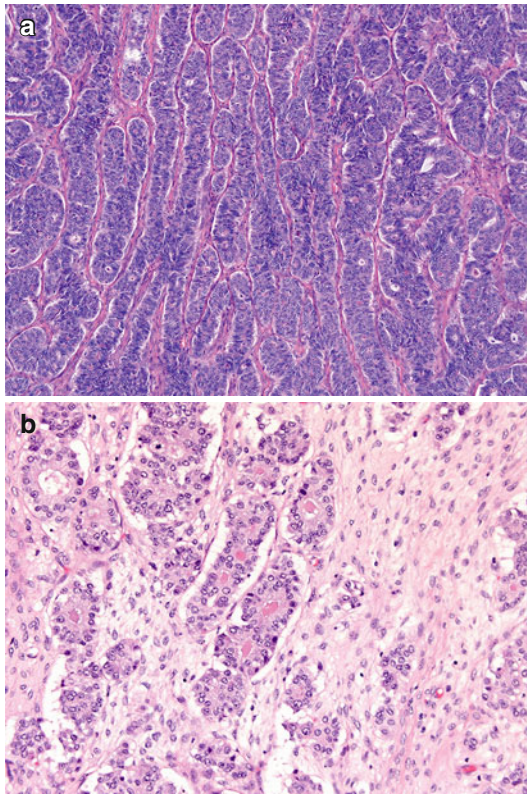

Fig. 11.11 Ovarian endometrioid adenocarcinoma with a sertoliform growth pattern. (**a**) Solid anastomosing tubules that are lined by columnar epithelial cells mimic a well-differentiated Sertoli-Leydig cell tumor. Very occasional small, glandular structures resembling hollow tubules are also seen. (**b**) In this case, small, hollow, anastomosing tubules are lined by low columnar epithelial cells and contain eosinophilic secretions. This also resembles a well-differentiated Sertoli-Leydig cell tumor

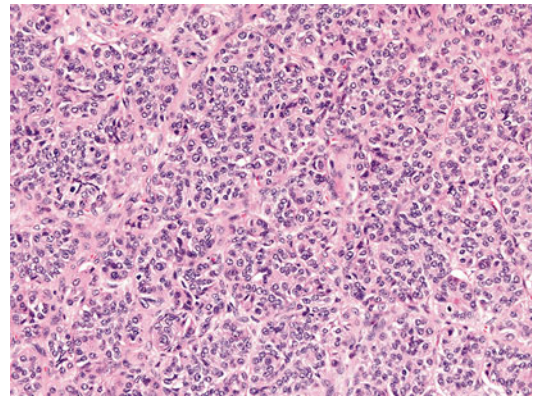

Fig. 11.12 Ovarian endometrioid adenocarcinoma with a granulosa cell tumorlike growth pattern. The tumor consists of islands and sheets of small epithelial cells with round vesicular nuclei and scanty cytoplasm. However, the typical nuclear grooves of a granulosa cell tumor are absent

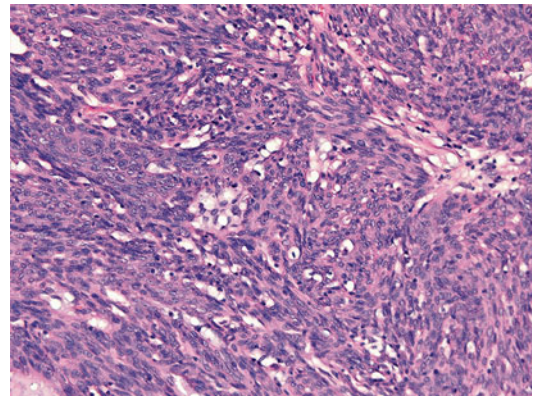

Fig. 11.13 Spindle-cell variant of ovarian endometrioid adenocarcinoma. This carcinoma is composed almost entirely of spindle cells with a fascicular and whorled arrangement. The spindle cells are a manifestation of squamous differentiation

Spindle-Cell Variant [45]

In this variant, 50 % or more of the tumor consists of spindle cells, which are a manifestation of squamous differentiation (Fig. 11.13). These raise the possibility of other spindle-cell tumors such as carcinosarcoma, tumors of probable Wolffian origin [46], and sarcomatoid/diffuse adult granulosa cell tumor. The spindle-cell component may be nested, diffuse, whorled, or arranged in interlacing fascicles with nuclear palisades which resemble schwannoma. Endometrioid glands are usually present but may be difficult to recognize because of their intimate intermingling with the spindle-cell component. The nuclear grade and mitotic index of the spindle cells tend to be the same as that of the glandular cells, i.e., low. This is a helpful differentiating feature from carcinosarcoma, which shows marked cellular pleomorphism with high-grade nuclear features and brisk mitotic activity. A heterologous malignant stromal component in the form of rhabdomyosarcoma, chondrosarcoma, or osteosarcoma may be present. Immunohistochemistry is helpful in

the differential diagnosis – see below. Caution should be exercised when grading tumors with a dominant spindle-cell component because the spindle cells are thought to represent a metaplastic process; this component should be excluded when assessing the percentage of solid growth in these tumors.

Clear Cell Variant

Secretory changes (in the form of subnuclear or supranuclear vacuolation) may be present in the columnar epithelial cells of ovarian endometrioid adenocarcinomas, or vacuolated, glycogenated squamous cells may be present in the squamous component. When such changes are only focal, they do not cause diagnostic difficulty. However, when widespread they may raise the possibility of a clear cell carcinoma, and concern may be heightened by the finding of endometriosis; endometriosis is associated not only with the development of ovarian endometrioid adenocarcinoma but also with ovarian clear cell adenocarcinoma [20]. Silva and Young [47] described a clear cell variant of endometrioid adenocarcinoma in which the cytoplasmic appearance ranged from "foamy to empty." The location of the clear cytoplasm was variable, and the cell nuclei were basal, central, or apical. The uniform cytomorphology of the secretory variant of endometrioid carcinoma was absent, as were the typical tubulocystic, solid, and papillary patterns of clear cell carcinoma. The immunoprofile of endometrioid carcinomas with a clear cell morphology is the same as that of the usual type of endometrioid ovarian adenocarcinoma, albeit that the intensity of staining is reduced. The expression of estrogen receptors may be absent in the clear cell areas but is present in non-clear cell areas, which are almost always present in such variants.

Other, Uncommon Variants

Ciliated cell [48] and oxyphilic [49] variants (Fig. 11.14) of ovarian endometrioid adenocarcinoma have been described, as has a rare variant with an α-fetoprotein-immunopositive component of yolk sac tumor [50].

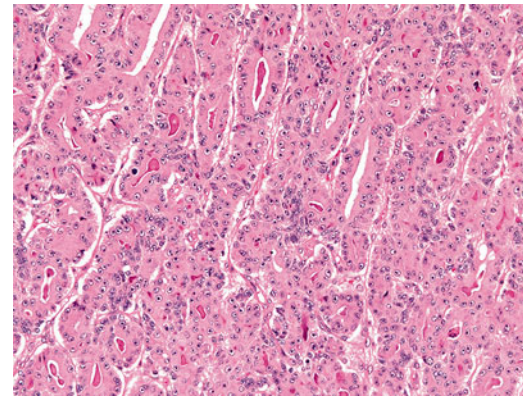

Fig. 11.14 Oxyphilic variant of ovarian endometrioid adenocarcinoma. The glands in this variant are lined by large cells with abundant eosinophilic cytoplasm. The cells in this example have relatively large vesicular nuclei and prominent nucleoli, but mitotic activity is low

Immunoprofile of Ovarian Endometrioid Adenocarcinoma [51]

Endometrioid ovarian adenocarcinomas, like endometrioid endometrial adenocarcinomas, may co-express vimentin (Fig. 11.15) and epithelial markers (cytokeratins including CK 7 and EMA) and show nuclear staining for β-catenin, particularly in areas with prominent squamous/morular metaplasia [52]. Like uterine endometrioid tumors, ovarian endometrioid tumors may have mutations of the β-catenin gene [53].

Endometrioid ovarian adenocarcinomas (and other primary Müllerian adenocarcinomas of the ovary) usually have a CK7-positive/CK20-negative immunoprofile, express CA125 and B72.3, and show nuclear staining for PAX8 (a transcription factor that has a role in the development of the Müllerian system). PAX8 is expressed by all ovarian epithelial tumors except mucinous adenocarcinomas [54]. Typically endometrioid ovarian adenocarcinomas express both estrogen and progesterone receptor (ER and PR) (Fig. 11.16) [55].

Low-grade endometrioid adenocarcinomas show only focal and patchy immunoreactivity for p16 and p53 and weak or absent labeling for WT1. However, high-grade endometrioid tumors (those with a cribriform or glandular pattern) may show overexpression of p53. Strong and

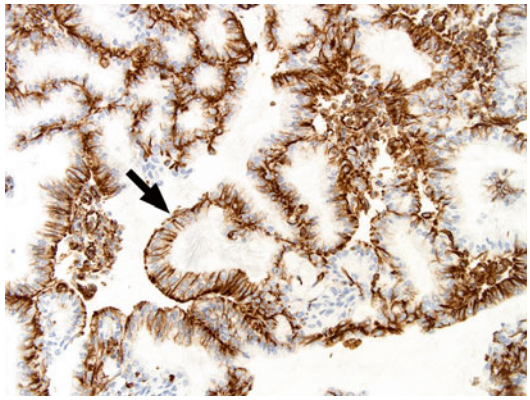

Fig. 11.15 Ovarian endometrioid adenocarcinoma. Lateral membrane labeling for vimentin (*arrow*) is typical of endometrioid tumors

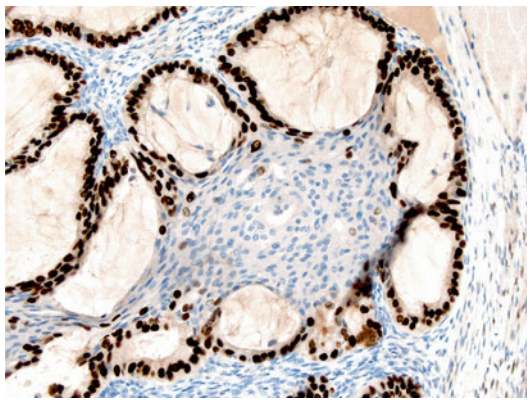

Fig. 11.16 Ovarian endometrioid adenocarcinoma. These tumors typically express hormone receptors. In this example the nuclei are strongly labeled with antibody to PR. Note that the antibody has not labeled the squamous morule

widespread WT1 and p16 expression is seen in high-grade serous carcinomas; weak expression of WT1 may be present in high-grade endometrioid adenocarcinomas.

A small proportion of ovarian endometrioid adenocarcinomas may express sex cord-stromal markers (inhibin or calretinin) [56]. If luteinized stromal cells are present, these too express sex cord-stromal markers. CEA may be demonstrable in up to 30 % of tumors, but TTF-1 and CDX-2 are absent. CD99 immunopositivity may occasionally be seen in endometrioid ovarian adenocarcinomas [57, 58].

Pathologists should be aware that squamous morules show strong expression of CD10

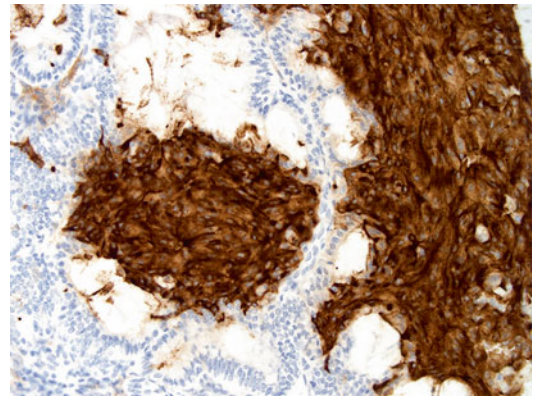

Fig. 11.17 Ovarian endometrioid adenocarcinoma with squamous morules which show strong expression of CD10

(Fig. 11.17). This can be misleading when CD10 immunohistochemistry is carried out to identify the endometrial stromal component of background endometriosis [59].

Differential Diagnoses (Table 11.2)

Sex Cord-Stromal Tumors

The hollow tubules in the sertoliform variant of ovarian endometrioid adenocarcinoma resemble Sertoli cell tumor, and the presence of stromal luteinization in some cases mimics well-differentiated Sertoli-Leydig cell tumors. More conventional areas of endometrioid adenocarcinoma are usually present, and the identification of squamous morules and an adenofibromatous background is helpful in confirming an endometrioid origin. The expression of CK7, EMA, ER, and PR supports an ovarian endometrioid adenocarcinoma origin, as does the lack of expression of sex cord-stromal markers (See section on Immunoprofile of Ovarian Endometrioid Adenocarcinoma above).

Adult granulosa cell tumors and ovarian endometrioid adenocarcinomas arise in a similar age group, and, because ovarian endometrioid adenocarcinomas occasionally include large islands of small epithelial cells and dilated glandular structures, the two types of tumor may be confused. A lack of both nuclear grooves and the varied architectural patterns of adult granulosa cell tumor favor ovarian endometrioid adenocarcinoma.

Table 11.2 Differential diagnosis

Primary endometrioid ovarian adenocarcinoma variant	Differential diagnosis	Morphological features that favor ovarian endometrioid origin	Immunohistochemistry that favors ovarian endometrioid origin
Sex cord-like or sertoliform endometrioid adenocarcinoma	Adult granulosa cell tumor (AGCT)	Confirmatory endometrioid features[a]	CK 7 +
		Typical endometrioid carcinoma in the background	EMA +
		Lack of nuclear grooves	CA125 +
		Range of architectural growth patterns of AGCT	ER and PR +
			Inhibin –
			Calretinin –
	Sertoli cell or Sertoli-Leydig tumor	Patients with endometrioid ovarian tumors are usually older	CK 7 +
		Confirmatory endometrioid features[a]	EMA +
		Typical endometrioid carcinoma in the background	CA125 +
			Inhibin –
			Calretinin –
Spindle-cell variant of endometrioid adenocarcinoma	Carcinosarcoma	Confirmatory endometrioid features[a]	WT1 –
		Low-grade cytological features in spindle-cell component	EMA + in spindle cells
		Similar mitotic index in glandular and squamous elements	p53 –
		Lack of heterologous sarcomatous component	
	FATWO	Confirmatory endometrioid features[a]	EMA +
		Lack of "sievelike" architecture in reticulin-stained sections	ER and PR +
			Inhibin –
			CD10 –
	Diffuse/sarcomatoid adult granulosa cell tumor	As for AGCT above	See above
High-grade, poorly differentiated endometrioid adenocarcinoma	High-grade ovarian serous carcinoma	Confirmatory endometrioid features[a]	WT1 –
		Lack of slit-like glandular spaces and complex papillary architecture	p16 –
		Tall, stratified columnar cells with uniform atypia	
		Lack of threefold variation in nuclear size	
		Mitotic rates usually not >10 in 10 ×40 objective fields	
Clear cell variant of endometrioid adenocarcinoma	Primary clear cell ovarian adenocarcinoma	Lack of papillary, tubulocystic, and solid growth patterns	HNF1 β –
		Lack of prominent stromal hyalinization and hyaline globules	ER +
		Tall columnar, not cuboidal cells	Vimentin + (lateral membrane)
		Lack of cytological atypia	

[a]Confirmatory endometrioid features
1. Morphological similarity to endometrium and endometrial, endometrioid uterine tumors
2. Metaplastic changes, most commonly of squamous/morular type
3. Associated features: endometriosis, preexisting benign or borderline endometrioid ovarian neoplasm

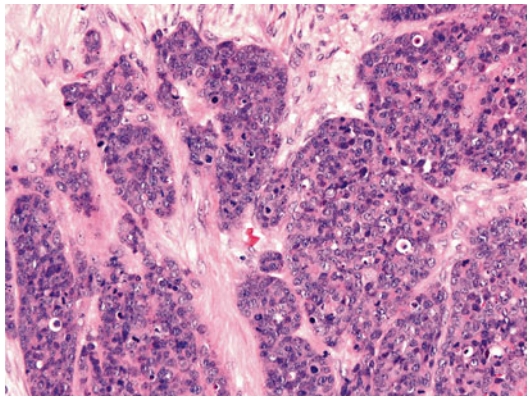

Fig. 11.18 Undifferentiated ovarian endometrioid adenocarcinoma. The adenocarcinoma has a solid, nested growth pattern and shows severe nuclear atypia, high mitotic activity, and apoptosis. This appearance mimics high-grade serous ovarian carcinoma

Occasionally the spindle-cell variant of ovarian endometrioid adenocarcinoma mimics sarcomatoid adult granulosa cell tumor. Reticulin silver impregnation will highlight the typical nested, reticulin-poor groups of granulosa cells, and areas of typical granulosa cell tumor or ovarian endometrioid adenocarcinoma may be present. "Confirmatory endometrioid features" (see above) are again helpful to confirm a diagnosis of ovarian endometrioid adenocarcinoma. As for other sex cord-stromal tumors, expression of CK7, EMA, ER, and PR supports an ovarian origin, whereas expression of sex cord-stromal markers (inhibin and calretinin) supports a diagnosis of adult granulosa cell tumor.

High-Grade Serous Carcinoma

Undifferentiated or poorly differentiated endometrioid ovarian adenocarcinoma (Fig. 11.18) may mimic high-grade serous carcinoma, especially when the carcinoma has a sheetlike growth pattern without a trabecular architecture or gland formation. A focal glandular component is usually present (if an obvious well-differentiated component is present in the background of an undifferentiated tumor component, this represents a dedifferentiated endometrioid adenocarcinoma). Features that favor poorly differentiated endometrioid adenocarcinoma over serous carcinoma are (1) smooth, rounded luminal gland contours as opposed to the irregular, slit-like contours of serous glands and (2) tall, stratified columnar cells that are uniformly atypical. In serous carcinomas, the tumor cells are usually cuboidal and show severe cytological atypia with nuclear size variation that is at least threefold. The pleomorphic nuclei contain prominent nucleoli and have mitotic rates in excess of 10 in 10 ×40 objective fields. Secretory or mucinous differentiation and "confirmatory endometrioid features" such as squamous metaplasia, endometrioid adenofibroma in the background, and endometriosis also support an endometrioid ovarian origin. Expression of p53 may be seen in both high-grade endometrioid and serous carcinomas, but expression of WT1 [60, 61] and p16 points toward a serous origin.

Carcinosarcoma

A prominent spindle-cell appearance as a result of confluent squamous differentiation may mimic carcinosarcoma. Endometrioid glands are usually present but may be difficult to recognize because they may be small, tightly packed, or compressed between the spindle cells. Carcinosarcomas typically contain pleomorphic, highly atypical spindle cells, with increased mitotic activity and atypical mitotic figures. A heterologous component may also be present. Typically, the spindle-cell component of endometrioid adenocarcinoma is of low nuclear grade, the same as that in the glandular component. An adenofibromatous background, areas of adenocarcinoma with more typical squamous/morular metaplasia, and a background of endometriosis all support a primary endometrioid ovarian adenocarcinoma. EMA and cytokeratin immunoreactivity are present in both the spindle and glandular cells, and p53 is not usually demonstrable immunohistochemically in low-grade endometrioid adenocarcinomas which show this growth pattern, whereas p53 expression is usually present in carcinosarcomas.

Ovarian Clear Cell Carcinoma

Extensive clear cell change in endometrioid ovarian adenocarcinoma may mimic clear cell carcinoma. Areas of typical endometrioid carcinoma

are usually present, and the typical tubulocystic, solid, and papillary patterns of clear cell carcinoma are absent. Stromal hyalinization, a feature that may be prominent in clear cell carcinomas, is not usually seen, and the intracytoplasmic hyaline globules of clear cell carcinoma are absent. Endometriosis may be present in both clear cell and endometrioid adenocarcinoma, but other "confirmatory endometrioid features" such as squamous metaplasia and endometrioid adenofibroma in the background may be helpful to confirm an endometrioid origin. Clear cell carcinomas are HNF-1β positive and do not express ER and PR.

Female Adnexal Tumor of Probable Wolffian Origin (FATWO)

Although FATWOs usually originate from the broad ligament, they may occasionally arise in the ovary. These tumors include a mixture of tubular, pseudotubular structures, and spindle cells. If the spindle-cell component predominates, a spindle-cell variant of endometrioid ovarian adenocarcinoma may enter into the differential diagnosis. The spindle-cell component in both tumors is cytologically bland, but the presence of other "confirmatory endometrioid features" is helpful, as is immunohistochemistry and the demonstration of the "sievelike" architecture of FATWO in reticulin-stained sections. FATWOs express inhibin and CD10, but not EMA, ER, or PR.

Metastatic and Synchronous Adenocarcinomas (See Also Chap. 16)

Metastatic colorectal tumors are sometimes misdiagnosed as primary endometrioid adenocarcinomas because of their cribriform, glandular architecture; the smooth, rounded luminal gland profiles; and the tall, stratified, uniformly atypical columnar cells that line the neoplastic glands. Colorectal metastases may present as an ovarian mass which may lead to the suspicion of a tumor of primary ovarian origin. Features that suggest metastasis include bilaterality, nodular growth pattern, ovarian surface involvement, lymphovascular invasion (especially in the ovarian hilar region), segmental or garland-like necrosis (which is often described as "dirty" necrosis as it includes cellular debris), and discordant high-grade cytological features in the presence of a low-grade architectural pattern. The presence of "confirmatory endometrioid features" favors a primary endometrioid adenocarcinoma. Immunohistochemistry can be useful in this situation: metastatic colorectal adenocarcinoma is usually CK20, CEA-M, and CDX2 positive, but CK 7, CA125, ER, and PR negative.

Although metastatic endocervical adenocarcinoma is uncommon, it may present as a synchronous or a metachronous ovarian carcinoma. In the latter context, ovarian mucinous tumor (borderline or malignant type) is usually suspected, but rarely metastatic mucin-poor cervical adenocarcinoma may resemble endometrioid adenocarcinoma. As in the case of other metastatic tumors, bilaterality, a nodular architecture, and lymphovascular invasion may provide clues to the provenance, and the presence of "confirmatory endometrioid features" supports a primary endometrioid adenocarcinoma. Endocervical adenocarcinomas show the same coordinate cytokeratin immunoprofile (CK7 positive, CK20 negative) as ovarian endometrioid adenocarcinomas, but detection of p16 or of HPV will help to confirm the diagnosis.

A diagnosis of metastatic endometrioid endometrial adenocarcinoma is favored by deep myoinvasion and lymphovascular invasion in the uterine tumor, involvement of the fallopian tube, small ovarian tumor size, bilateral ovarian involvement, multinodular growth pattern and lymphovascular invasion in the ovary, ovarian surface involvement, and the absence of "confirmatory endometrioid features" associated with the ovarian tumor.

Tumor Genetics

The most commonly identified genetic abnormalities in endometrioid ovarian carcinomas are

somatic mutations of the β-catenin gene (*CTNNB1*) and *PTEN*. *CTNNB1* mutations are reported in up to 50 % of cases. β-Catenin can be detected immunohistochemically within tumor cell nuclei in >80 % of endometrioid ovarian tumors. Endometrioid ovarian tumors with *CTNNB1* mutations are usually of low stage with a good prognosis [62]. Mutation of *CTNNB1* was reported in up to 90 % of borderline ovarian endometrioid tumors [63].

PTEN mutations are identified in up to 42 % of ovarian endometrioid adenocarcinomas [64, 65]. Loss of heterozygosity (LOH) on 10q23 is present in up to 43 % of endometrioid ovarian tumors, and in one study *PTEN* mutations were detected in almost half of those tumors with 10q23 LOH. Tumors with *PTEN* mutations are usually low grade and low stage which suggests that inactivation of *PTEN* is an early event in the endometrioid ovarian tumor pathway. The identification of 10q23 LOH in over half of endometriotic ovarian cysts and mutations of *PTEN* in approximately 20 % support the proposal that endometriosis is a precursor of ovarian endometrioid adenocarcinoma.

PIK3CA encodes the α catalytic subunit of phosphatidylinositol-4,5-bisphosphate 3-kinases, which have important roles in cell growth motility and proliferation. *PIK3CA* mutations were identified in 20 % of ovarian endometrioid and clear cell carcinomas [66]. *PIK3CA* mutations are present in high-grade ovarian endometrioid tumors (unlike *PTEN* and *CTNNB1* mutations which occur in low-grade tumors) [67]. In one recent study, *PIK3CA* amplification was predictive of resistance to chemotherapy [68].

TP53 mutations can be identified in >60 % of endometrioid ovarian tumors, more commonly in those of high grade [69]. More recently, somatic mutations in the *ARID1A* gene (which encodes BAF250a, a key component of the SWI-SNF chromatin remodeling complex) were identified in about one third to one half of ovarian endometrioid carcinomas [70, 71]. In 2 cases in one of the studies, *ARID1A* mutations were found in adjacent atypical endometriosis [70]. Mutations in this gene may therefore also be important in the early stages of the transformation of endometriosis into ovarian carcinoma.

Although microsatellite instability (MSI) is found in endometrioid endometrial adenocarcinomas, it occurs in <20 % of ovarian endometrioid adenocarcinomas [72–74]. Lynch syndrome (in which germ line mismatch repair gene mutations lead to MSI) may be associated with ovarian adenocarcinoma; non-serous ovarian carcinomas are overrepresented in these patients (in one study 35 % of ovarian tumors were endometrioid and 17 % clear cell) [75]. Ovarian carcinomas associated with Lynch syndrome present at a young age and are of low stage. However, the morphological criteria used to screen patients with uterine cancer for further Lynch syndrome testing (tumor-infiltrating lymphocytes, peritumoral lymphocytes, and dedifferentiated morphology) are not found in the ovarian tumors of patients with this syndrome [76].

Prognosis and Staging

The treatment of endometrioid ovarian adenocarcinomas is the same as for other ovarian carcinomas. Because these tumors tend to express ER and PR, they are amenable to hormonal manipulation with antiestrogens, tamoxifen, and progestogens. Hormonal therapy is particularly helpful if chemotherapy cannot be tolerated, in cases of recurrence, or when surgical options are limited by patient morbidity. Data on response rates to hormonal therapy are limited [77].

In ovarian carcinomas, the specific tumor cell subtype is an independent prognostic indicator [78, 79]. Gilks et al. [79] showed that 90 % of non-serous ovarian carcinomas were stage I or II at diagnosis, whereas serous carcinomas were significantly more likely to present with advanced disease (82 % of all stage III tumors were serous). Tumor grade was examined in the same study, and although the Silverberg grade was found to be a better prognostic indicator than the FIGO grade, both FIGO and Silverberg grades allowed patients to be subdivided into groups which had significantly different risks of relapse and survival [40]. Molecular abnormalities are associated

with specific tumor subtypes and are likely to guide future treatment.

In addition to presenting more often with early-stage disease, patients with endometrioid ovarian carcinoma have a better performance status overall, fewer patients at all stages present with ascites compared to patients with serous tumors, more patients with endometrioid tumors are amenable to optimal debulking, and the progression-free survival is better [3]. After receiving platinum-based chemotherapy, optimal tumor debulking and disease stage are the most important independent predictors of survival.

Although national and international data are available on the incidence, stages, and survival of ovarian carcinoma in general, there are only limited data available for endometrioid ovarian adenocarcinoma as a separate subset. The 1991 FIGO report (cited in Lee et al. [31]) indicated a 5-year survival rate of 78 % for patients with FIGO stage I ovarian endometrioid tumors, 63 % for stage II tumors, 24 % for stage III tumors, and 6 % for stage IV tumors. However, more recent data indicate that patients with stage I endometrioid ovarian tumors have a >90 % 10-year disease-specific survival compared with 70 % for clear cell and mucinous carcinomas and 40 % for serous tumors [79]. For stage II and III endometrioid ovarian carcinomas, the prognosis is better than for serous tumors of equivalent stage [3]. The presence of peritoneal keratin granulomas is of no prognostic significance [32, 33].

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Clear Cell Carcinoma of the Ovary

12

David W.M. Millan

Abstract

There is little known about the treatment of gynecological clear cell carcinomas, but there is a rapidly accumulating appreciation that they are a more diverse group of tumors than was previously known. The combination of conventional histological analysis of this variable group together with other biological parameters has given rise to an individualized approach to devising new treatments. However, we still rely on the recognition of their histopathological appearances which are described in this chapter.

Introduction

Ovarian clear cell carcinoma has been recognized as a distinctive histopathological entity since 1973 when the WHO published its classification of ovarian tumors [1]. Previously, it had been classified as a "mesonephroid" tumor of the ovary given its similarities to the pathological appearances of clear cell carcinoma of the kidney.

It is histologically and biologically distinct from other epithelial ovarian tumors and more recently has been recognized as being a heterogeneous entity including its etiology, appearances, and molecular profiling [2, 3]. A consistent observation, however, is its relative chemoresistance, in particular to platinum-based therapies [4–6].

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Clear cell carcinoma is a relatively uncommon type of epithelial ovarian cancer, accounting for approximately 6 % of epithelial ovarian carcinomas. Interestingly, it also shows a significant variation in incidence depending on ethnic background. It has been recognized that clear cell carcinoma is significantly more common in Asian countries, in particular, in Japan, where clear cell tumors constitute at least 20 % of epithelial ovarian cancers [7–9].The cause of this variation in incidence could have many sources. In particular, one potential variation was thought to be due to the different incidences of reporting by pathologists in different countries. However, recent experience from the international clinical trial organized by the Japanese Oncology Group under the auspices of the Gynecological Cancer Intergroup reveals that the diagnosis of clear cell carcinoma of ovary is a robust and consistent one with very little difference in the reporting profile of pathologists working in different countries (personal observation). Furthermore, the annual

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report of the Japanese cancer committee has demonstrated an increased incidence of clear cell ovarian carcinoma. It is now estimated that clear cell carcinoma may constitute over 25 % of ovarian carcinomas.

Clinical Presentation

The presentation of clear cell carcinoma of the ovary is distinct from that of the much more common serous carcinoma [10]. It usually presents as a single pelvic mass, confined to the ovary with no clinically apparent peritoneal metastases. There is a frequent association with thromboembolic events at presentation, and hypercalcemia is also recognized incidentally in some cases. Ascites is relatively uncommon, in contrast to serous tumors. Raised serum levels of Ca125 are variable, due in part to the close association with endometriosis which is also present in 50–70 % of cases [11–13]. The average age of presentation is 10 years younger in those tumors arising in endometriosis, and this also impacts on survival in these two groups. The overall survival in the endometriosis linked cases is 196 months in contrast to only 34 months where it is absent. This is also linked to the finding of an earlier stage at diagnosis in the endometriosis group [14]. The relationship of clear cell carcinoma to endometriosis is complex, with a large number of potential confounding factors including, among others, the definition and diagnosis of endometriosis. However, there are numerous cohort studies, some of which include large numbers of women, which do support the contention that there is a significant relationship between the two conditions [15].

Bilateral ovarian clear cell carcinomas are rare. In studies where lymph node evaluation was undertaken, it was found that occult nodal metastases were significantly less common with clear cell carcinoma (7.9 %) in comparison with serous carcinoma (13.6 %) [7]. Given this higher incidence of true early-stage disease confined to the ovary might suggest that the overall survival from stage I clear cell carcinoma is better than serous carcinoma. However, this is where the biology of clear cell carcinoma impacts on its behavior and emphasizes the difference between the two tumor types. There have been a number of conflicting studies with results claiming a similar prognosis with early-stage disease but a poorer outcome with more advanced disease, others suggest that there is no difference between the two groups [16]. The concluding study with the largest number of patients has been the SEER (surveillance epidemiology and the end results) data looking at 5-year survival for distinct histological groups of ovarian tumors. In this study, the behavior of 1,411 clear cell tumors was compared with 13,835 serous carcinomas. It was found that the survival of patients with clear cell carcinoma was poorer but only significantly with more advanced stage (III/IV) disease [7]. In view of the recognition that there are different types of both clear cell carcinoma and serous carcinoma, this data may be worth revisiting.

Macroscopic Appearances

The majority of clear cell carcinomas are unilateral and their frequent association with endometriosis means that they are frequently seen in association with each other. The diagnosis of a carcinoma is often a surprising finding to an unsuspecting clinician. The preceding history of endometriosis requiring operative treatment in a woman with long-standing symptoms should alert the operating clinician to the possibility of a coincident carcinoma.

The tumors occur either at an early stage as an isolated mass or alternatively at a more advanced stage with disseminated disease. There does tend to be a more distinct pattern of presentation with these two groups and this is of prognostic significance.

In the isolated discrete and often incidental diagnosis, there is an ovarian mass with a variable appearance. There may be the typical multicystic, hemorrhagic tumor with solid and cystic areas. In the cases of those with an adenofibromatous background, the solid areas are hard and have a more fibromatous appearance. This appearance warrants very careful sampling as atypical or the so-called borderline, and microinvasive carcinomas can be focal and therefore may be missed.

If there is easily recognizable, typical endometriosis, there may also be an unsuspected area of a more fleshy, soft, polypoid tissue in an otherwise cystic mass. Tumor tissue in this situation is more commonly of a pure, typical clear cell, carcinoma which frequently merges with benign epithelium into atypical areas and then frank carcinoma.

Histological Appearances and Classification

It should come as no surprise that a clear cell carcinoma of ovary need not necessarily be made up of cells with optically clear cytoplasm. There are a range of histopathological appearances with papillary, tubulocystic, oncocytic, and solid patterns. These different architectural patterns are usually admixed with each other. Clear cell carcinomas are not infrequently mixed with other histological appearances, most commonly endometrioid tumors. This is most likely because of its association with endometriosis, which is also significantly associated with endometrioid ovarian carcinomas. However, when a clear cell carcinoma does arise in this background, it is more common to see a gradation from endometriosis to atypical endometriosis to atypical clear cell differentiation and finally to clear cell carcinoma. It is relatively unusual to see clear cell carcinoma as part of an otherwise typical and predominant endometrioid carcinoma. Where there is a more genuine admixture of clear cell carcinoma with other histological appearances such as serous or mucinous differentiation, careful attention must be made to consider and exclude a malignant non-epithelial or stromal component as would be seen in a carcinosarcoma. These apparently mixed tumors must also be critically assessed as to whether they represent variants of the more typical and common serous tumors. In the recent Japanese Oncology Group chemotherapy trial, recruitment was limited to tumors showing an arbitrary figure of 50 % clear cell differentiation such cases were accepted together with the condition that there was no evidence of serous tumor. In practice, the majority of these apparently mixed tumors were rare and virtually all were finally regarded as high-grade serous tumors [17].

It has also become evident that rarely some clear cell carcinomas arise on a background of a clear cell adenofibroma (Figs. 12.1, 12.2, and 12.3). This is a rare benign usually solid tumor with biphasic architecture of dense fibrous stroma with discrete islands of microcystic and acinar

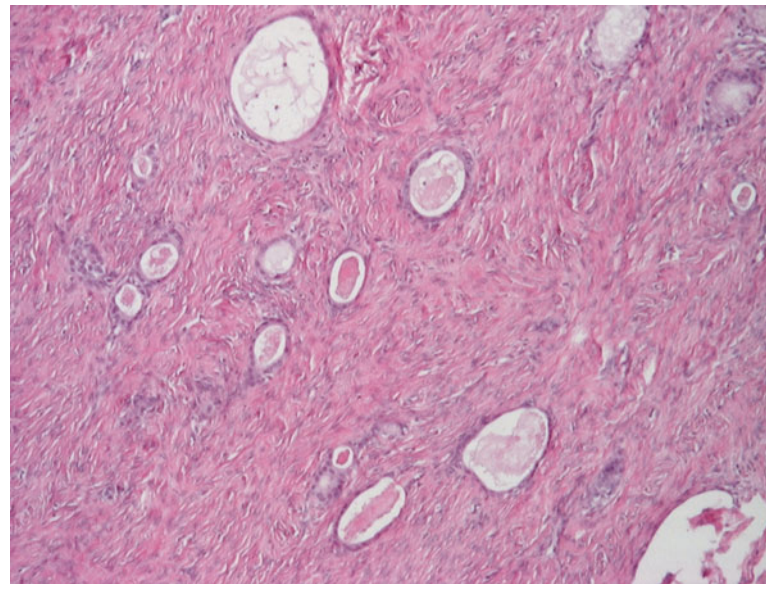

Fig. 12.1 Benign clear cell adenofibroma. A collection of simple tubulocystic structures lying in a typical dense ovarian fibromatous stroma

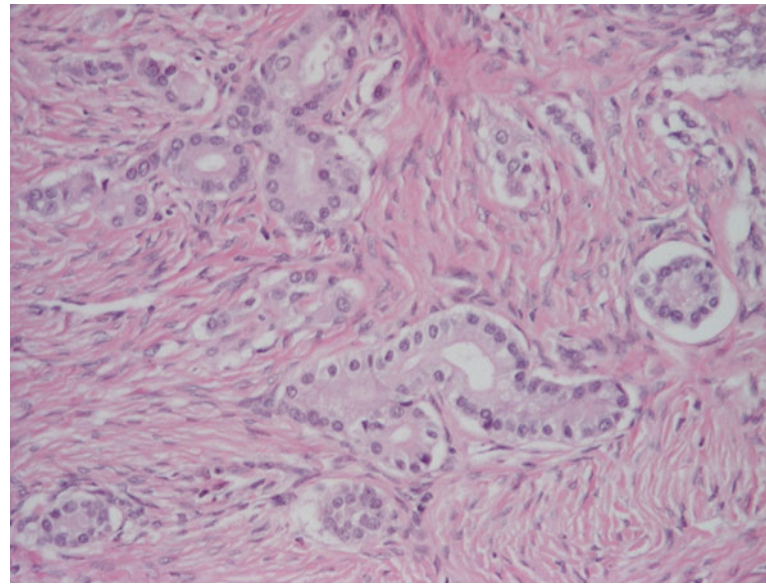

Fig. 12.2 Early stromal invasion in a clear cell adenofibroma. Minimally atypical tubular and cystic glands with a more complex architecture and with individual single and cords of cells infiltrating the stroma. In cases where this is difficult to identify, the use of a cytokeratin to identify and highlight the epithelium can be helpful

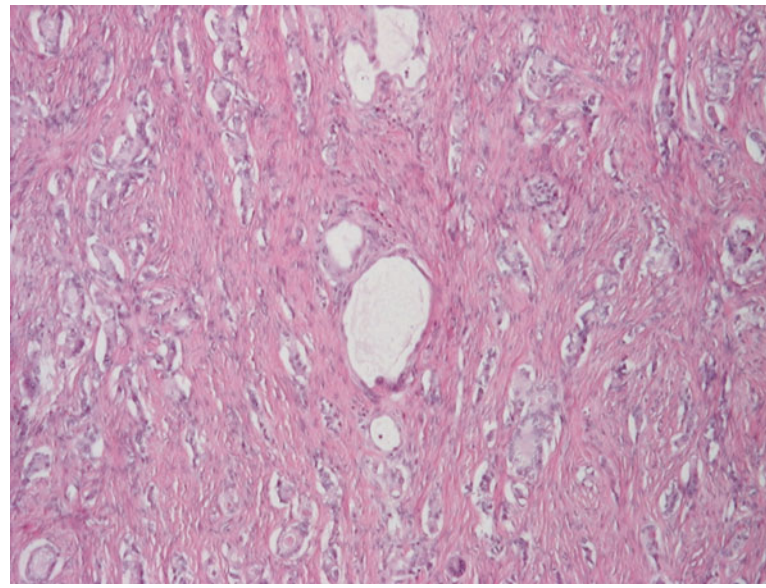

Fig. 12.3 Clear cell adenocarcinoma arising in an adenofibromatous background. A very complex and more diffuse pattern of stromal infiltration

epithelial groups which have a variable degree of cytoplasmic clearing. There is a gradation of atypia within the epithelial component. There are benign tumors with bland cytological appearances ranging to those with epithelial cells with atypical nuclei forming borderline lesions and with increasingly abnormal nuclei amounting to a clear cell adenocarcinoma [18]. Cytological atypia is one component, but it is also architectural atypia with the coalescence of cystic or tubular structures to form more complex solid or cribriform proliferations which enable the diagnosis of a frank carcinoma.

The second, even less common, appearance is infiltration of the stroma by individual abnormal cells. It is these more subjective assessments of the architecture which can make it difficult to give a robust and convincing diagnosis of malignancy rather than atypia or borderline changes. Further thorough sampling of the tumor mass

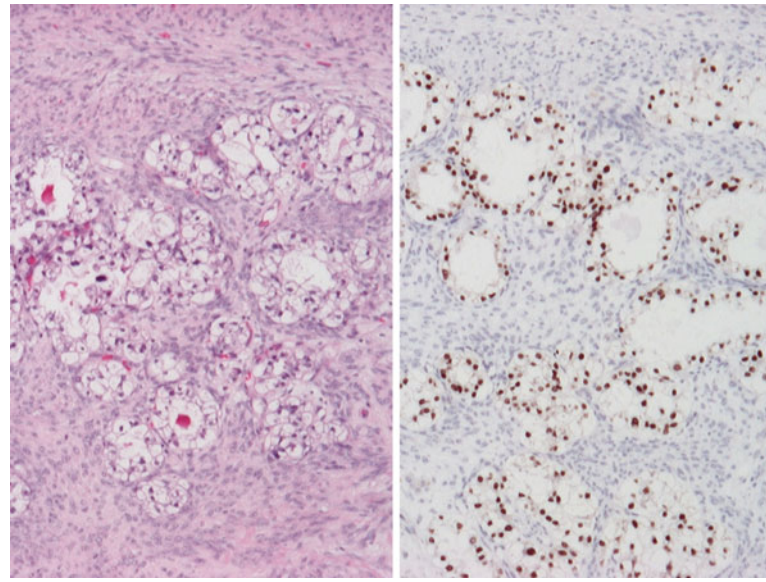

Fig. 12.4 A classic clear cell carcinoma with positive nuclear staining for hepatocyte nuclear factor 1 beta (HNF-1 beta)

may be helpful, often revealing more convincing evidence of malignancy which was originally suspected on the initial sections.

Immunohistochemistry has a limited contribution to the diagnosis of clear cell carcinoma. There is the usual pattern of cytokeratin expression with CK7 positivity and only rare and focal CK20 expression. It has certainly demonstrated that they are distinctive tumors with a relative lack of ER, WT1, and P53 expression. Expression of hepatocyte nuclear factor 1-beta (HNF-1beta) is one of the few more distinctive positive markers (Fig. 12.4). In a small series of 30 cases of clear cell tumors, all stained positively. Furthermore, associated endometriosis also showed positivity suggesting that its expression may indicate a premalignant change [19]. It has also been suggested that because HNF-1beta is involved in glycogenesis and glycolysis that its overexpression may be part of the cause of a clear cell appearance [20]. Where there is the unusual difficulty in distinguishing between serous and clear cell tumors, a diagnostic panel of HNF-1beta, ER, and WT1 has shown to be of use with serous tumors showing positivity with the latter two antibodies [3, 21, 22].

Immunohistochemistry can help in the unusual situation where other less common diagnoses enter into the differential diagnosis. Rare cases of metastatic renal cell carcinoma can be shown to be RCC and CD10 and PAX8 positive, although this latter antibody is also positive in a proportion of ovarian clear cell carcinomas.

If yolk sac tumor is being considered in the differential diagnosis, it is worth noting that clear cell carcinomas can rarely express AFP. CK7 and EMA expression in clear cell tumors distinguishes it from yolk sac tumors.

As expected from the higher incidence of early-stage disease, clear cell carcinomas have a relatively low proliferation index so that staining with Ki-67 and cyclin A is low [23].

Gene expression studies have also confirmed that clear cell carcinoma and serous tumors are distinct and different. One interesting observation is the higher incidence of mutations in PTEN, the tumor suppressor gene, which has been compared with the evolution of endometriosis into endometrioid carcinoma and clear cell carcinoma [24].

Gene expression of clear cell carcinoma has also been compared with serous, endometrioid, and renal clear cell tumors and has emphasized that there are some similarities between clear cell carcinomas arising in different organs [25].

In the light of gene profiling together with emerging specific and very expensive, targeted therapies, it is now the pathologist's role to not

just make a diagnosis and define a stage but to identify those subsets of tumors which will be more amenable to treatment with these new agents. There are a number of evolving strategies with treatment using agents which inhibit specific tyrosine kinases and the PI3K-AKT-mTOR-HIF-mediated pathway [26]. It is estimated that there are mutations in the latter pathway in the order of 40 % of cases of clear cell carcinoma. The other significant pathway includes mutations in a tumor suppressor gene ARID1A which occurs in approximately 50 % of cases.

Histopathological Appearances

Grading of Clear Cell Carcinomas

The recommendation of the World Health Organization (WHO) is that this tumor should not be given a formal grade. There is evidence, as noted above, that the stage of the tumor and, therefore, ability to optimally debulk the tumor are the most significant prognostic indicators. Furthermore, where there has been an attempt to grade using more conventional approaches, no significant association with outcome has been identified [27].

Grading of ovarian carcinomas is currently a fluid entity, and there is good evidence married with molecular analysis that pathologically we will have to take other clinicopathological features and molecular profiling data into account. It is certainly apparent that there are clear differences in the evolution of both clear cell and serous and possibly other epithelial ovarian tumors.

Biologically, there does appear to be two groups of clear cell ovarian carcinomas; in its broadest definition, there are those early tumors which are genuinely confined to the ovary and a second group of advanced aggressive disseminated tumors. The former group has a mixed pattern of pathological appearances and apparent origins. The latter blend into areas of both recognizable clear cell patterns and poorly differentiated carcinomas.

It is not known if there is a biological difference between these two patterns of disease.

This complex background raises the issue of features which are worthy of directed treatment and the need for tissue sampling throughout the evolution of the disease in order to characterize its molecular profile. There are still significant questions with regard to the clinicopathological definitions of clear cell carcinomas. These are particularly pertinent in the context of clinical trials where it is paramount that there is consistency and robustness in the diagnosis of a specific tumor group.

When should a tumor be considered a “clear cell tumor”? In the recent Japanese trial, an arbitrary and pragmatic definition was that more than 50 % of the tumor should consist of clear cell carcinoma and its variants. This proved to be a consistent and workable definition. This has been further refined to include the absence of any evidence of serous differentiation within the tumor as such lesions are likely to represent variants of high-grade serous tumors.

A further question is as to what is the significance of clear cell change in other tumor types. This is a common problem. The most frequent issue is the mistaken identification of squamous metaplasia. This is because of the cytoplasmic clearing which is seen and which can be mistaken for clear cell differentiation. In contrast to clear cell carcinoma, in areas of squamous metaplasia, there is a lack of high-grade nuclear changes and lack of a complex architecture. In more challenging cases, immunohistochemistry reveals a pattern of positive staining with CD10 and CDX2 within squamous morules.

What is a borderline clear cell tumor? This pertains to two entities: the borderline or atypical clear cell adenofibroma and the atypical endometrioid tumor or endometriosis. This latter feature can be seen in association with endometriosis where there is a gradual transformation of a benign epithelium into cytologically atypical areas and in turn into areas with an atypical clear cell appearance.

In the former entity, there is an obvious adenofibromatous appearance. There are well-defined, usually acinar, collections of very variably epithelial clear cells set within a dense fibromatous stroma. In addition, there is cytological atypia with nuclear enlargement, irregularity, and more prominent nucleoli. At a low-power assessment, there is also architectural complexity. Careful

attention of a well-sampled tumor may also reveal early invasion of individual cells into the surrounding stroma, indicative of a microinvasive tumor. Strict measurement criteria of such lesions in the context of clear cell tumors is controversial, but in practice the criteria used for serous carcinomas is usually applied. This is certainly of questionable relevance given the relative very good prognosis of early-stage clear cell tumors and the unknown significance of microinvasion in a clear cell lesion.

Classical Histological Patterns

Tubulocystic Pattern (Figs. 12.5 and 12.6)

The tumor has architecture of clefts and spaces lined by cells with a distinctive appearance. They can have a cuboidal shape with clear cytoplasm and the usual high-grade, open vesicular nuclei with prominent nucleoli. Alternatively, the cell can take on the characteristic (but not diagnostic) hobnail appearance where the cytoplasm of the

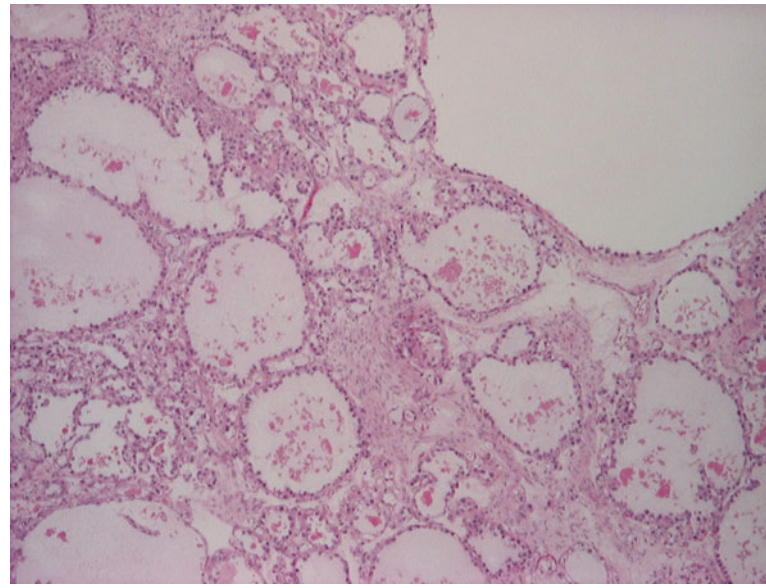

Fig. 12.5 Clear cell carcinoma with a tubulocystic appearance. There are variable cystic spaces lined by both hob-nail cells and clear cells. The typical hyalinised stroma is also prominent

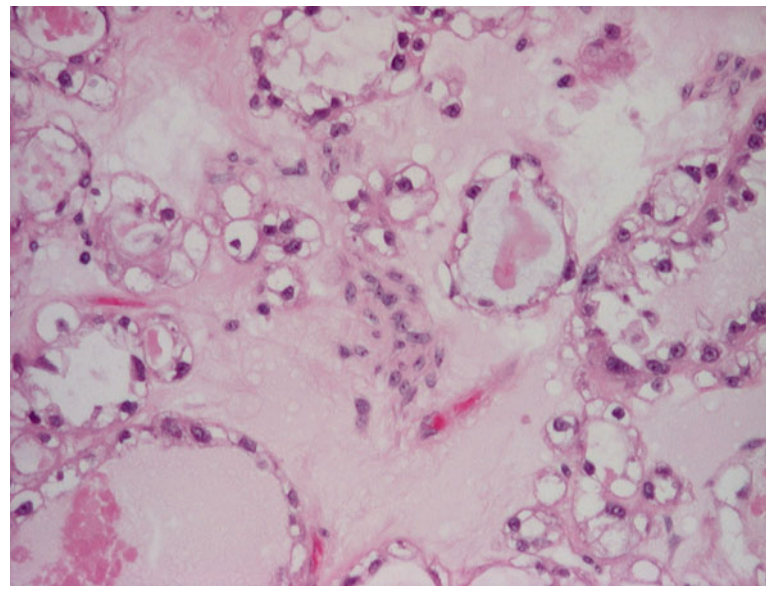

Fig. 12.6 Clear cell carcinoma with a typical tubulo-cystic appearance and easily recognisable cells with clear cytoplasm. The blood vessel off centre is distant from the surrounding epithelium in contrast to the close apposition typically seen in renal carcinomas

cell protrudes into the luminal space and is largely occupied by the nucleus.

Papillary Pattern (Figs. 12.7, 12.8, 12.9, and 12.10)

The papillae tend to be broad with prominent vascular cores with recognizable stromal tissue. Over the surface of these wide papillae are cells similar to those described above with their distinctive vesicular nuclei. The papillary pattern is usually admixed with areas of tubulocystic architecture. In contrast to the papillae of serous or endometrioid carcinomas, there is only a layer of 1 or 2 cells thick. In serous and endometrioid tumors, there are multiple layers of cells, and with serous tumors in particular, there are the occasional large bizarre pleomorphic nuclei. Nuclear pleomorphism to the degree seen in serous tumors is only rarely present in clear cell carcinomas.

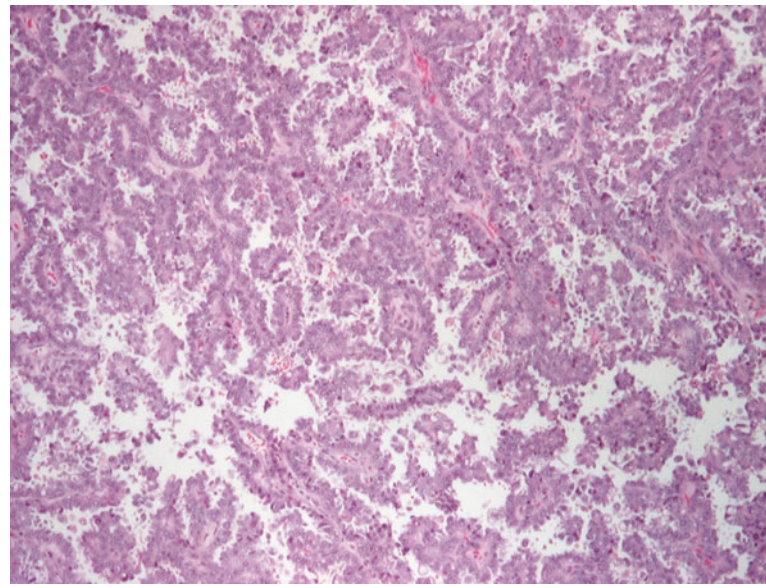

Fig. 12.7 Clear cell carcinoma with a prominent papillary architecture. In this low power image there is also an easily identifiable hob-nail appearance to the lining epithelium

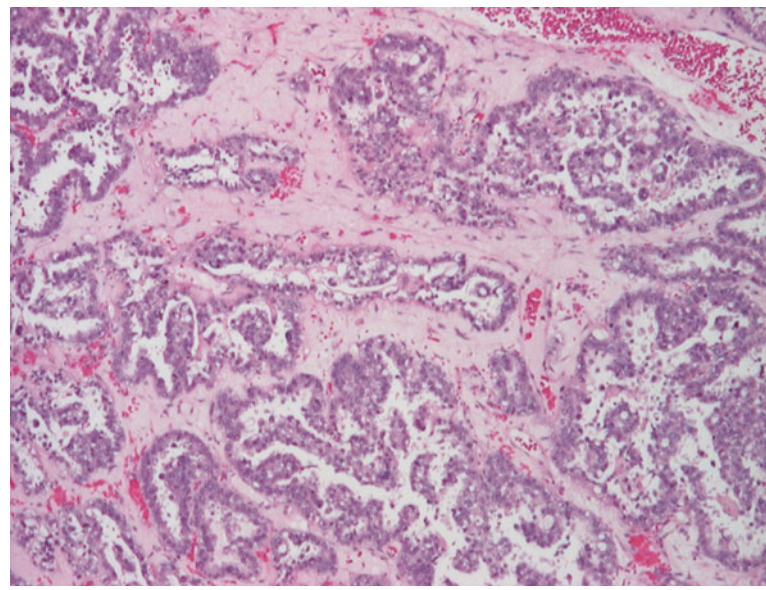

Fig. 12.8 Clear cell carcinoma with a tubulo-papillary architecture. There is a typical hyalinised stroma and a mixture of cells with clear and non-clear cytoplasm

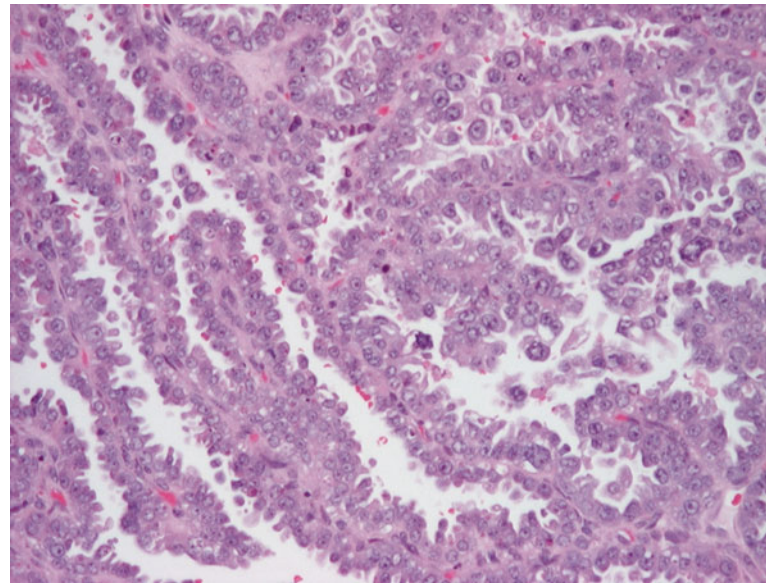

Fig. 12.9 Clear cell carcinoma with no conventional clear cells but with prominent hob-nail cells where each epithelial cell nucleus protrudes into the lumen above the surface of the surrounding cells, the so called hob-nail appearance

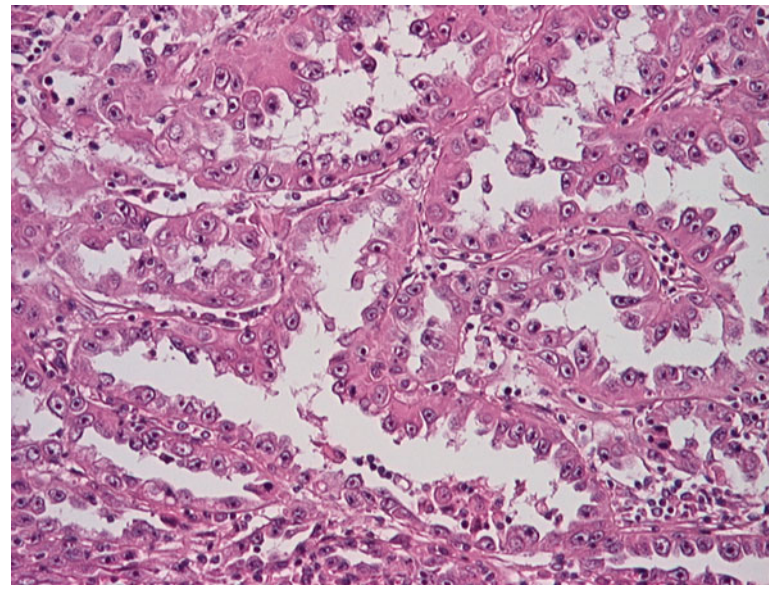

Fig. 12.10 Clear cell carcinoma with a tubulopapillary architecture. There are moderately pleomorphic nuclei which have very prominent central nucleoli

Solid Pattern (Figs. 12.11 and 12.13)
There is a cohesive sheet of polygonal cells with prominent cell boundaries and typical clear cytoplasm. As in all the other subtypes, there are only moderately pleomorphic nuclei with prominent nucleoli. It is with this pattern that the distinctive intercellular hyalinized eosinophilic globules are more readily identified. These eosinophilic, AFP negative globules are a useful feature, consistent with a clear cell phenotype, but they cannot be used as a basis for a conclusive diagnosis. Although it may occupy only a small area of tumor, this classic clear cell appearance is present in virtually all clear cell carcinomas but may only be evident with thorough sampling. In contrast to renal clear cell carcinomas, there is a much less prominent thin-walled vascular network in ovarian tumors. There is the rare exception where the solid tumor arises from a clear cell adenofibromatous background where

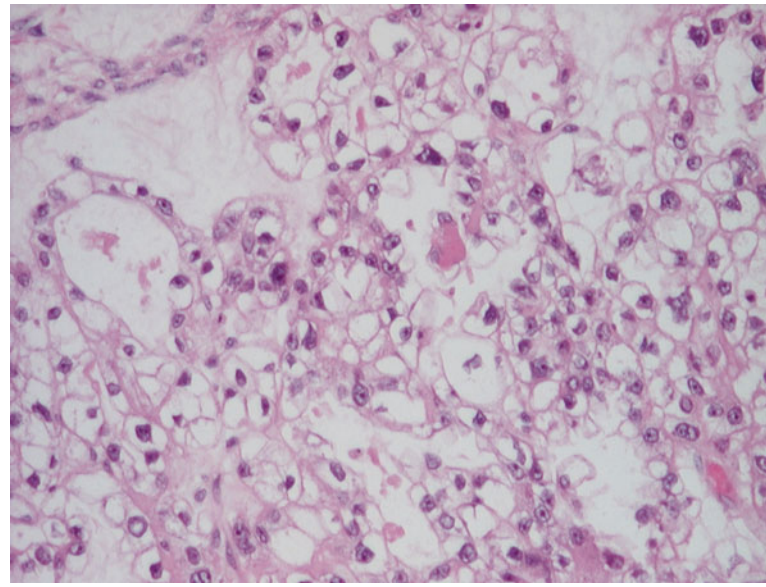

Fig. 12.11 A classic clear cell adenocarcinoma. Typical tumour cells with clear cytoplasm with vesicular nuclei with prominent nucleoli arranged in a solid and acinar arrangement. Intercellular eosinophilic bodies are easily seen and there is only an inconspicuous vascular network

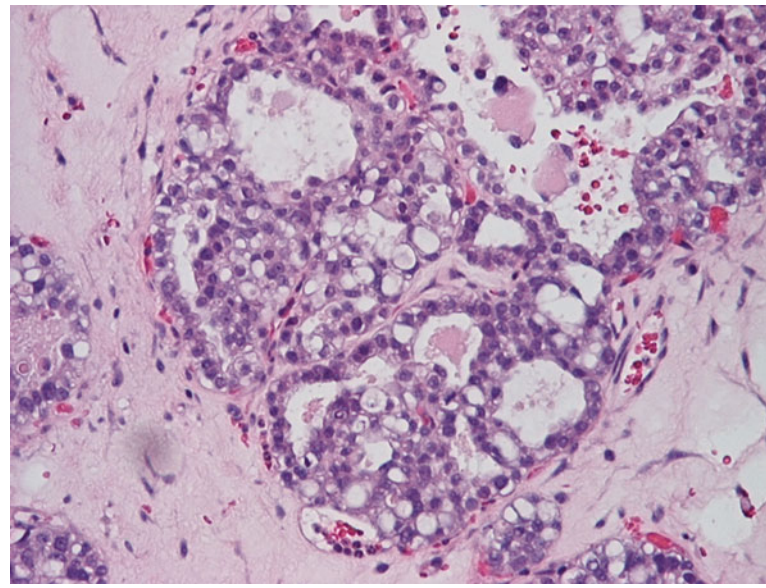

Fig. 12.12 Clear cell carcinoma with a mixture of clear and non-clear cells

classic clear cell differentiation is much less evident; as stated previously, it is the combination of atypical cytology and complex or infiltrative architecture which form the basis of a diagnosis of carcinoma.

Oxyphilic Tumors (Figs. 12.12 and 12.13)
Rarely the tumor may be made up of cells with moderately abundant eosinophilic slightly granular cytoplasm giving rise to the oxyphilic variant. As with other clear cell carcinomas, there is often a similar component showing the more common solid, papillary, or tubulocystic appearances. The more typical clear cell appearances may only make up a small proportion of the tumor.

Differential Diagnoses

Serous carcinomas of high-grade type have typical solid areas with slit or crack-like spaces together with variable numbers of large cells with large,

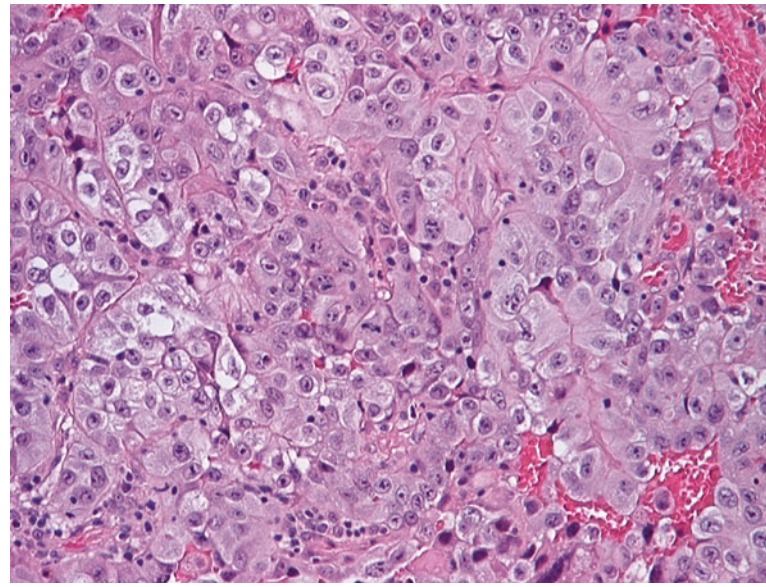

Fig. 12.13 Clear cell carcinoma. There is a predominance of cells with abundant oxyphilic cytoplasm. In comparison with the preceeding images there is a consistent similarity with the vesicular nuclei and prominent central nucleoli. There is also a suggestion of cytoplasmic clearing in some cells. Where this is the dominant pattern a careful search for more conventional areas of clear cell change helps in confirming the diagnosis

bizarre, pleomorphic nuclei. Cytoplasmic clearing giving rise to clear cells and eosinophilic intercellular globules are rarely seen in serous tumors. If this proves morphologically problematic, the distinctly different immunohistochemical profile in these two apparently similar tumors can be helpful in establishing the definitive diagnosis.

Immunohistochemistry of high-grade serous tumors typically shows diffuse, strong positive staining for P53, WT1, and ER and a high proliferation index with Ki67.

In contrast, the findings in a clear cell carcinoma with immunohistochemistry are negative immunoreactivity with WT1, ER, and p53 and a low proliferation index with Ki 67.

Low-grade serous tumors have a much more consistent and uniform delicate papillary architecture with small, only mildly pleomorphic nuclei and rarely show cytoplasmic clearing. WT1 and ER are also typically positive, in contrast to clear cell tumors.

Endometrioid carcinomas with prominent squamous metaplasia can give rise to a clear cell appearance giving rise to the mistaken impression of a mixed tumor. Staining for either CD10 or CDX2 can positively identify these squamous morules and so rule out a clear cell carcinoma. In view of the rare production of AFP by clear cell carcinomas, yolk sac tumors may enter into the differential diagnosis. AFP is only rarely positive in clear cell carcinomas and this is also only focal in nature. Yolk sac tumors are typically negative with CK7 and EMA, in contrast to clear cell carcinomas.

Renal cell carcinomas do have a superficial resemblance to their ovarian counterpart and can in rare circumstances enter into the differential diagnosis. Renal tumors have a distinctive network of thin-walled blood vessels intimately associated with tumor cells. CD10 and RCC stains are typically positive in renal tumors but not in ovarian tumors.

Prognosis and Treatment

Early-stage clear cell carcinoma appears to be a different disease entity with a relatively good rate of survival. The rare clear cell adenofibromas are essentially benign. This also includes the cytologically atypical or so-called borderline tumors.

There is some evidence to suggest that ex-adenofibromatous clear cell carcinomas are relatively less aggressive [27, 28]. There is evidence from several studies, including a meta-analysis, which show that clear cell carcinoma has a poorer prognosis than non-clear cell tumors. However, in comparison to

serous carcinomas, there is only a significant difference with advanced stage disease (stage 111 and 1 V) where clear cell histology imparts a worse prognosis [29]. The 5-year survival for clear cell carcinoma is in the order of 85.3 % for stage 1 disease, but this falls to 17.5 % for stage 1 V disease [7]. Precise histopathological definition is lacking in these trials, and their validity is therefore in doubt.

The main mode of treatment is surgical with the ongoing debate as to the value of lymphadenectomy. In part, this is because retroperitoneal lymph node involvement is less common in clear cell carcinoma.

There are a number of ongoing trials aiming to determine the optimal chemotherapy regimen as platinum resistance is also relatively more common in this group, and specific pathway-inhibiting drugs are now available [26], and this offers a new line of investigation but only in partnership with precise pathological definition.

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13 Undifferentiated, Transitional, Mixed, and Other Epithelial Tumors of the Ovary

Sanjiv Manek

Abstract

There are a number of less common but important benign and malignant epithelial ovarian tumors to be aware of in addition to the commonly encountered serous, endometrioid, mucinous, and clear cell tumors. These less frequent tumors include those formed of transitional cells, squamous cells, mixed epithelial cells, mixed epithelial and mesenchymal cells, and a large group in which the epithelial cells are undifferentiated. The most important of these are the undifferentiated cell group which includes the aggressive carcinomas with or without a neuroendocrine component as well as an associated malignant or benign epithelial lesion. Apart from the benign transitional tumors, which are often incidental findings, the unusual epithelial ovarian tumors make up less than 3 % of all ovarian tumors. All of these will be considered in turn with emphasis on their diagnostic features, differential diagnoses, ancillary methods for diagnosis, and common pitfalls. There will be mention of diagnostic difficulties with frozen sections in this group of tumors.

There are a number of less common but important benign and malignant epithelial ovarian tumors to be aware of in addition to the commonly encountered serous, endometrioid, mucinous, and clear cell tumors [1]. These less frequent tumors include those formed of transitional cells, squamous cells, mixed epithelial cells, mixed epithelial and mesenchymal cells, and a large group in which the epithelial cells are undifferentiated. The most important of these are the undifferentiated cell group which includes the aggressive carcinomas with or without a neuroendocrine component as well as an associated malignant or benign epithelial lesion. Apart from the benign transitional tumors, which are often incidental findings, the unusual epithelial ovarian tumors make up less than 3 % of all ovarian tumors [2]. All of these will be considered in turn with emphasis on their diagnostic features, differential

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diagnoses, ancillary methods for diagnosis, and common pitfalls. There will be mention of diagnostic difficulties with frozen sections in this group of tumors.

Undifferentiated Carcinomas

A diagnosis of undifferentiated carcinoma should only be made once all recognizable specific features and the possibility of metastatic carcinomas or lymphomas have been excluded [3–6]. This can only be achieved by thorough sampling and a careful search for distinguishable features and use of immunohistochemistry. Hence, any high-grade solid carcinoma showing even a hint of glandular differentiation, papillae formation, or the presence of psammoma bodies should be classified as one of the more common carcinomas and not as undifferentiated carcinoma. Most of those undifferentiated carcinomas with gland and papillae formation are best classified as high-grade serous carcinomas. In the literature, there is mention of the allowable presence of glands or papillae in 5 % of undifferentiated carcinomas, but this is subjective and dependent on sampling.

Undifferentiated carcinomas can be further classified into five main subtypes: small cell carcinoma (hypercalcemia type (SmCCHT)); small cell carcinoma, pulmonary type (SmCCPT); non-small cell neuroendocrine carcinoma; undifferentiated carcinoma associated with endometrioid carcinoma; and undifferentiated carcinoma, not otherwise specified (NOS). All of these are rare and only account for less than 1 % of invasive carcinomas, and the SmCCHT and those associated with endometrioid carcinomas are the most common. The 2006 FIGO annual report states that the undifferentiated carcinoma group makes up 5.4 % of all ovarian carcinomas [7]. This wide variation in the quoted incidence rates ranging from 1 to 5.4 % reflects the variation in the diagnostic criteria of undifferentiated carcinomas.

Undifferentiated carcinomas other than the SmCCHT occur in the older age group and usually present at an advanced stage. 15 % are bilateral. The SmCCHT is typically seen in the third decade. There are no special presenting symptoms or signs. The raised calcium seen in cases of SmCCHT is often recognized retrospectively and occurs in 60 % of cases. Macroscopically, the undifferentiated carcinomas are large and solid tumors with hemorrhage and necrosis frequently encountered. Some, particularly the undifferentiated carcinoma, NOS, are similar in appearance to high-grade serous carcinomas. There are variations in microscopic features and in the immunohistochemistry as outlined below.

Small Cell Carcinomas, Hypercalcemia Type (SmCCHT)

This rare malignancy occurs at a young age, peaking in the third decade. It presents with abdominal pain and/or distension and only a few have symptoms related to the hypercalcemia. The hypercalcemia is likely due to binding of PTH-like peptide to PTH receptors [8]. The tumors can be large (up to 20 cm) and comprise both solid and cystic components. It is composed of small round cells with scant cytoplasm which are generally closely packed, often with nuclear molding. Within the same tumor, there can be areas of spindled cells, nests, cords, clusters, and diffuse sheets of cells, with the diffuse pattern predominating in most cases. Follicle-like cystic areas are characteristic and these are of variable shapes and sizes (Fig. 13.1a), most containing eosinophilic secretions. The cells at the edges of these spaces are compressed and often angular and nuclear molding is most apparent in these areas (Fig. 13.1b). The nuclei are hyperchromatic with chromatin granularity and prominent nucleoli. The mitotic index is generally high. There is a large cell variant of this subtype of undifferentiated carcinoma (seen in 50 % of cases) in which the nuclear features are similar but the cells have more cytoplasm which is eosinophilic (Fig. 13.2). If vacuolation is present in the cytoplasm, the cells can appear rhabdoid. In the small cell variant, there is scant stroma, but in the large cell variant, there can be variable amounts of myxoid stroma.

SmCCHT can be associated with mucinous neoplasms (carcinoma in 9 %, benign tumors in up to 3 %) [9]. The mucinous element is usually endo-

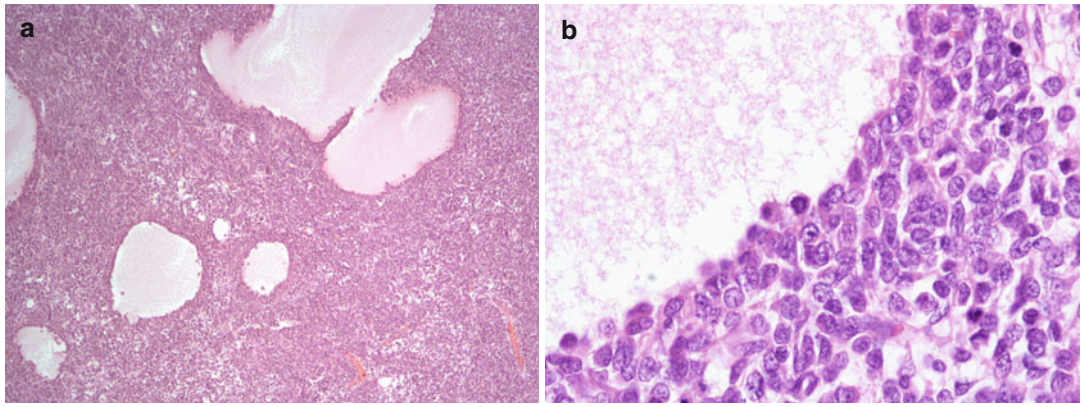

Fig. 13.1 (**a**) Small cell carcinoma, hypercalcemia type. Low power to show the diffuse sheet-like arrangement of cells with irregular follicle-like spaces. © Illustration Services, Cellular Pathology, OUH. (**b**) Small cell carcinoma, hypercalcemia type. High power to show nuclear molding, often appreciated close to a follicle-like space. Note the high mitotic activity and cells with scant cytoplasm (© Illustration Services, Cellular Pathology, OUH)

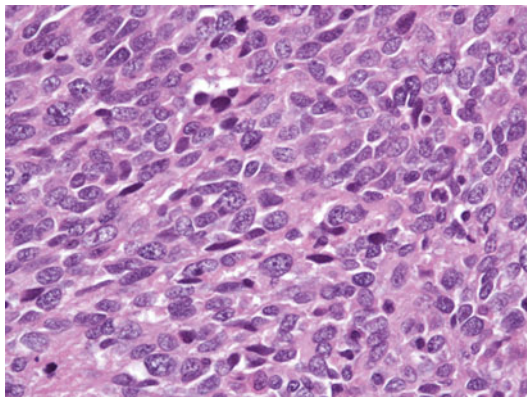

Fig. 13.2 Large cell variant of small cell carcinoma, hypercalcemia type. The cells are larger with more cytoplasm which is eosinophilic

Table 13.1 The main immunohistochemical profile of SmCCHT

Positive	Negative
WTI (diffuse, nuclear)	TTF 1
CK 8/18	Chromogranin
P53	Desmin
CD10	Melan A
EMA	CD99
CAM 5.2	Inhibin
NSE	PLAP
Calretinin	OCT4
Vimentin	HP
Synaptophysin (+/−)	HCG
PTH-related hormone (+/−)	CK5
	CD117

cervical in type and, in some cases, can adopt a signet ring cell morphology. Table 13.1 includes the main immunohistochemical profile of SmCCHT.

Some undifferentiated carcinomas may contain foci of choriocarcinoma in which case HCG can be positive in these areas. This immunopanel allows distinction from the main differential diagnoses which include the diffuse pattern of adult granulosa cell tumor and the juvenile granulosa cell tumor (both inhibin positive); the latter also shows a diffuse growth of small dark cells with irregular follicle-like spaces. However, the juvenile granulosa cell tumor has a more distinct nodular growth pattern with fibrous septa separating the nodules on a background that appears fibrothecomatous. Other differential diagnoses include desmoplastic round cell tumors (desmin positive), metastatic melanoma (melan A positive), transitional cell carcinomas, and other small round, blue cell tumors as well as lymphomas. Poorly differentiated Sertoli-Leydig cell tumors can also look like SmCCHT. One also has to exclude metastatic small cell carcinoma from the lung (TTF1 positive). Diffuse pattern dysgerminomas can be confused with SmCCHT, but dysgerminomas usually contain fibrous septa and lymphocytes in the background, and they are positive for OCT4, PLAP, and CD117. Other

undifferentiated small cell carcinomas, particularly the SmCCPT, can also be part of the differential diagnoses. However, the SmCCPT occurs in older women and there is no hypercalcemia. They are also often associated with other epithelial tumors such as endometrioid carcinomas. SmCCPT are TTF1 positive and vimentin negative. Rarely primitive neuroectodermal tumors have to be excluded, and the large cell variant SmCCHT can be confused with rhabdomyosarcoma which typically show positive immunoreactivity for desmin, myogenin, and Myo D1.

The prognosis of SmCCHT is very poor (33 % survival at 5 years), and this is related to age at presentation, calcium levels, size of tumor, and absence of large cells. Surgery is the main form of treatment as radiotherapy and chemotherapy are generally ineffective.

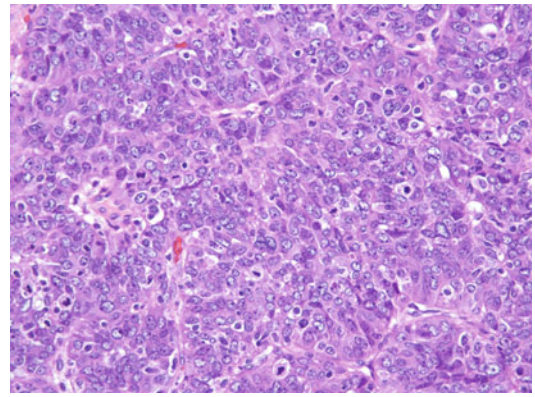

Fig. 13.3 Undifferentiated carcinoma associated with low-grade endometrioid carcinoma. High-power view of an area of undifferentiated carcinoma with oval cells containing vesicular nuclei with prominent nucleoli. Elsewhere, there was low-grade endometrioid carcinoma (© Illustration Services, Cellular Pathology, OUH)

Undifferentiated Carcinoma Associated with Low-Grade Endometrioid Carcinoma

In some endometrioid adenocarcinomas of the ovary, there may be undifferentiated carcinoma elements which can sometimes predominate [10]. These comprise sheets of loose cells which are generally small but can have large cell variants. These cells are oval or round with vesicular nuclei and prominent nucleoli (Fig. 13.3). The cells can be nested and separated by thin fibrous septa. The large cell variant often comprises cells with vacuolated cytoplasm. As in the SmCCHT, a rhabdoid appearance may be seen in such large cells. The differentiated portions of these carcinomas are usually low-grade. It is thought that the undifferentiated elements represent areas of dedifferentiation in the low-grade endometrioid carcinomas [11]. The undifferentiated areas do not have glands or a trabecular pattern and the nuclear features are somewhat more aggressive in appearance than in the differentiated parts. A grade 3 endometrioid adenocarcinoma composed of solid tumor will contain some glands and trabeculae and have nuclear features more typical of endometrioid carcinoma.

Immunohistochemically, these cells are focally positive for EMA and CK18. They may also be focally positive for synaptophysin and chromogranin.

The main differential diagnoses to consider are grade 3 endometrioid adenocarcinomas which show more diffuse positivity with cytokeratins and EMA; neuroendocrine carcinomas which are generally diffusely positive for synaptophysin and chromogranin; metastatic endometrioid carcinoma; lymphoma, endometrial stromal tumors; rhabdoid tumors; and plasmacytomas.

These are aggressive malignancies with low survival rates. Even small components of undifferentiated carcinomas in otherwise low-grade endometrioid carcinomas can impart a worse prognosis.

Small Cell Carcinomas, Pulmonary Type (SmCCPT)

This is a rare primary malignancy in the ovary [12]. It comprises small to medium cells usually in diffuse sheets often with prominent nuclear molding. There are no follicle-like spaces present unlike the SmCCHT. These carcinomas are often associated with endometrioid carcinomas and Brenner tumors. Table 13.2 shows the immunohistochemical profile of SmCCPT.

The main differential diagnoses are the other undifferentiated carcinomas of the ovary and true

Table 13.2 The immunohistochemical profile of SmCCPT

Positive	Negative
Cytokeratins (dot positive, especially CK20)	Vimentin
EMA	Desmin
NSE	CD117
Chromogranin	Inhibin
TTF 1	

metastases from a small cell carcinoma of the lung. Morphological and immunohistochemical features can help to differentiate these from each other. They can be hormonally active and cause paraneoplastic syndromes. These are aggressive malignancies with poor survival rates. Surgery and chemotherapy are the main forms of treatment.

Non-small Cell Carcinoma, Neuroendocrine Type [13, 14]

These are very rare, rapidly growing malignancies and comprise medium to large cells which are usually arranged in sheets but can have a nested pattern. Vague follicle-like spaces can be present but are not as frequent as in the SmCCHT. They can be associated with endometrioid or mucinous carcinomas in over 50 % cases as well as teratomas and borderline mucinous tumors. Immunohistochemically, they are positive with synaptophysin and often with chromogranin. Before making a diagnosis of a primary neuroendocrine carcinoma, the possibility of metastases from other more common sites needs to be excluded. Large cell neuroendocrine carcinomas can arise in mature cystic teratomas [15].

Undifferentiated Carcinomas, Not Otherwise Specified (NOS)

This is also a rare primary malignancy in the ovary and is diagnosed when the other subtypes of undifferentiated carcinomas have been considered and excluded. These generally comprise medium to large cells with a high mitotic index. There is no specific pattern to the architecture and usually there are no associated tumors or lesions. These carcinomas bear the same immunoprofile as other more common epithelial malignancies with positive staining for CK7, CA125, EMA, and CAM5.2 and often with WT1. Hence, the main differential diagnosis is high-grade serous carcinoma displaying a solid growth pattern. Metastatic carcinoma also needs exclusion as another differential diagnosis.

Although all undifferentiated carcinomas pursue an aggressive course, adequate sampling for microscopic examination is very important to rule out the main differential diagnoses which may have better prognoses. Furthermore, immunohistochemistry will also aid in excluding other malignancies in cases of undifferentiated carcinomas with vague features.

Frozen Sections of Undifferentiated Carcinomas

When ovarian masses are sent for frozen section diagnoses, it may not be possible to subtype undifferentiated carcinomas [16] unless there are classical follicle-like spaces visible when a diagnosis of SmCCHT can be suggested. All undifferentiated carcinomas will appear as high-grade malignancies on frozen sections and will therefore have a large differential diagnosis, particularly when the carcinomas are made up of large cells. The differential diagnoses to consider include lymphomas, undifferentiated sarcomas, melanoma, rhabdomyosarcoma, high-grade serous carcinoma, poorly differentiated squamous cell carcinomas, dysgerminomas, and poorly differentiated granulosa cell tumors in which the classical patterns are absent. Metastatic carcinomas also have to be considered in frozen sections.

Transitional Cell Tumors [17]

These are generally referred to as Brenner tumors and comprise 2 % of epithelial tumors. They are composed of urothelial cells and even possess some true urothelial differentiation within the benign tumor category. The exact origin of the tumor cells is unknown but recent evidence suggests a possible origin from transitional cell nests at the tubal-mesothelial junction [18]. The most com-

mon is the benign Brenner tumor. Borderline and malignant Brenner tumors are rare. In this group of tumors is also included the transitional cell carcinoma (TCC) mainly because of the pattern of tumor growth and the architecture. TCCs of the ovary do not show urothelial differentiation [19] despite a growth pattern similar to a papillomatous TCC in the urinary tract or an inverted papilloma of the sinonasal tract. TCCs are now regarded to be variants of high-grade serous carcinomas and, as such, a better designation for these tumors is high-grade serous carcinoma with transitional-like differentiation. The morphology and immunoprofile of TCCs of the ovary are different from malignant Brenner tumors with the latter showing areas of benign and borderline Brenner tumor in the background and some urothelial differentiation.

Benign Brenner (Transitional Cell) Tumors

These occur mainly in the sixth decade but can be seen at any age. They are often small tumors found incidentally. The larger tumors are usually unilateral and can present as masses but do not usually manifest with hormonal symptoms. The small tumors can range from a few millimeters to 2 cm and can be multifocal. The larger tumors can be as large as 10 cm in diameter and sometimes more. Macroscopically, they are mainly solid with a firm, white/yellow, often trabeculated, cut surface. Sometimes they are lobulated or can appear like uterine fibroids. Cystic areas are not common but occasional tumors can be predominantly cystic (uni- or multiloculated) due to degeneration. A few solid tumors can be considerably calcified and difficult to cut.

Microscopically, the tumors comprise distinct epithelial nests of variable shapes and sizes (Fig. 13.4a) within a fibromatous or thecomatous stroma which is variably calcified and can be considerably hyalinized (Fig. 13.4b) and sometimes luteinized. Osseous metaplasia can occur. The epithelial cells are uniform with little pleomorphism and absent mitoses. They are multilayered and generally ovoid in shape. Their nuclei are vesicular and also ovoid and may contain grooves rendering a "coffee bean" appearance. Nucleoli are not prominent. When the nests are large, they can be cystic and contain mucin or eosinophilic secretions. Two types of metaplasia can occur in benign Brenner tumors. Squamous metaplasia is often seen in cystic tumors and, if extensive, can appear as epidermoid cysts. Mucinous metaplasia is more common and variable in degree. When extensive, these tumors are referred to as metaplastic Brenner tumors [20]. Conversely, 20 % of benign mucinous tumors contain Brenner tumors. In some mucinous tumors with a Brenner component, the latter is more than just an incidental nodule, and such tumors can also be called metaplastic Brenner tumors or

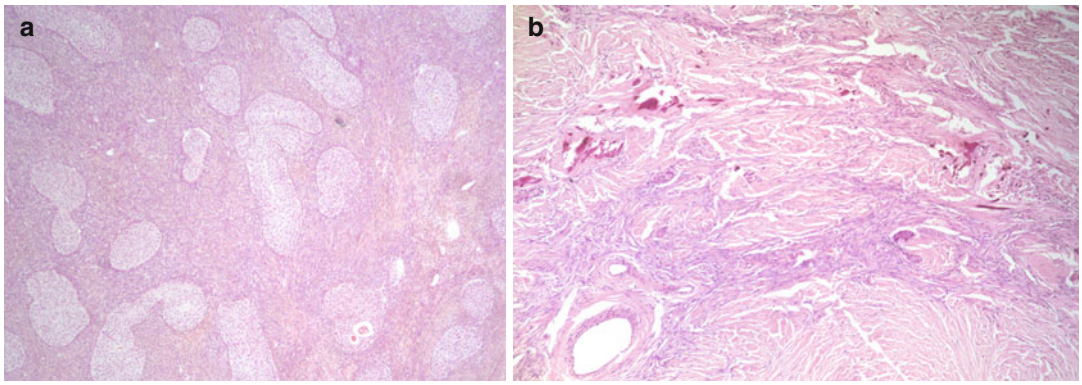

Fig. 13.4 (**a**) Benign Brenner tumor. Distinct nests of transitional cells in a fibromatous stroma. Some nests have small cystic spaces. (**b**) Benign Brenner tumor. Only an occasional small transitional cell nest is seen in a markedly hyalinized and calcified stroma

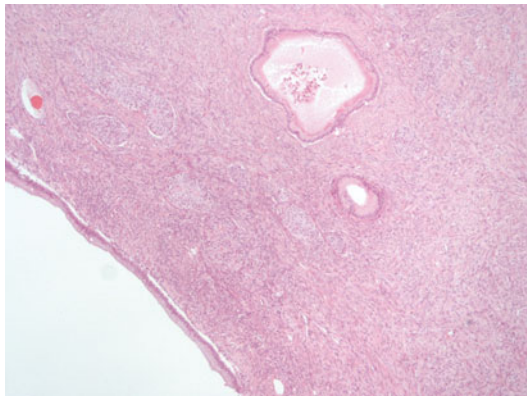

Fig. 13.5 Metaplastic Brenner tumor. Note the close proximity of mucinous cysts and transitional cell nests

Table 13.3 The immunohistochemical profile of benign Brenner tumors

Positive	Negative
CK7	CK20
CA 125	Inhibin
EMA	WT1
P63	Calretinin
CEA	p53
CA 19.9	EGFR
Thrombomodulin	Ras protein [22, 23]
Uroplakin 3 (focal)	

mixed mucinous-Brenner tumors which tend to occur in older women and are usually unilateral. In 30 % of metaplastic Brenner tumor cases, the Brenner tumor component is intermingled with the mucinous component. When the mucinous component in a mixed mucinous-Brenner tumor becomes prominent, it may give rise to an appearance of a coexisting Brenner tumor with a mucinous cystadenoma (Fig. 13.5). It is possible that this is a source of primary mucinous ovarian tumors that do not arise in teratomas [21].

Fifty percent of Brenner tumors are associated with peritoneal Walthard's rests seen in the paratubal region. These rests also comprise transitional epithelium and can become markedly cystic. Benign Brenner tumors can also be found in conjunction with serous cystadenomas, dermoid cysts, struma ovarii, and carcinoids. Table 13.3 includes the main immunohistochemical profile of benign Brenner tumors.

Borderline Brenner Tumors [24]

These are rare tumors and usually unilateral. They are not associated with peritoneal and omental implants. They occur in middle-aged to elderly women and often present as a pelvic mass, generally being larger than benign Brenner tumors. They can measure up to 30 cm in diameter and are usually cystic. Sectioning of the cystic areas can reveal nodular masses and polypoid areas, and in the wall, there can be firm fibrous areas which often represent background benign Brenner tumor elements. Microscopically, the characteristic feature is of broad papillae projecting into cystic locule(s) representing epithelial proliferation. These papillae are lined by multilayered transitional epithelium, and the overall pattern resembles low-grade papillary TCC of the urinary bladder. Growth into the wall is represented by small closely packed nests of transitional epithelium. Invasion does not appear to occur, but there can be focal features of intraepithelial or in situ carcinoma. These include marked cytological atypia and a high mitotic index. However, the majority of borderline Brenner tumors contain cells with only mild to moderate cytological atypia. In most cases, areas of benign Brenner tumor can be seen in the background. Immunohistochemically, borderline Brenner tumors share the same immunoprofile as their benign counterparts.

Malignant Brenner Tumors

These are characterized by the presence of benign and borderline Brenner tumor elements in the background. They are rare and usually present as low-stage malignancies although one-third of cases do have extraovarian spread at presentation. They account for less than 0.5 % of all ovarian carcinomas. They tend to occur in middle-aged and elderly women. They are usually not as large as their borderline counterparts but can be cystic like them. They may have areas of hemorrhage and necrosis. Microscopically, they are composed of abundant, irregular, angulated nests of atypical transitional cells arranged in a haphazard

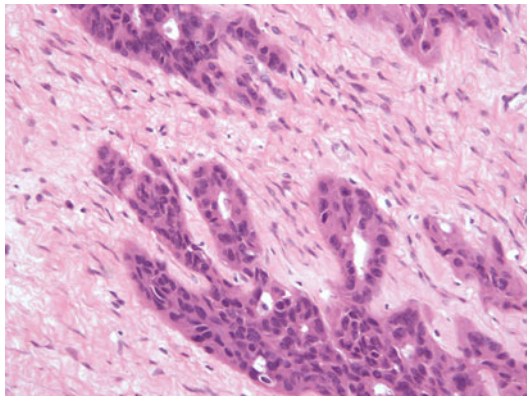

Fig. 13.6 Malignant Brenner tumor. High-power view to show small angulated nests of cells with hyperchromatic nuclei with infiltrative features. There is no desmoplasia in this field

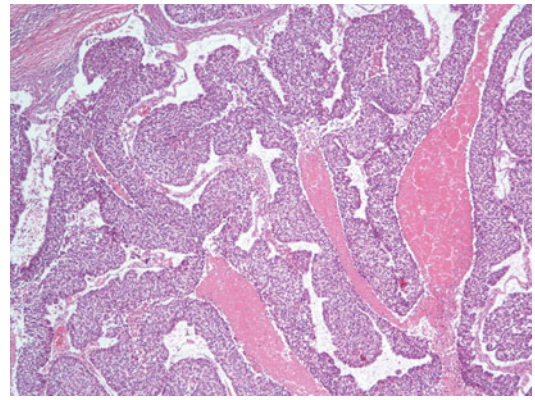

Fig. 13.7 Transitional cell carcinoma. Inverted papilloma-like features. The cells are uniform and transitional in appearance. Apoptosis and necrosis are evident

pattern with infiltrative features (Fig. 13.6). The stroma is usually fibrous but not necessarily desmoplastic [25]. Comedo-type necrosis can be seen in the infiltrative nests. At low power, they can be mistaken for metastatic carcinoma showing an irregular pattern of infiltration. Benign and borderline Brenner tumor elements are present in the background and the appearance is often of a spectrum of disease from benign to malignant. Papillary projections into cystic locules can be seen. There is moderate cytological atypia and brisk mitotic activity. Mucinous, squamous, or spindle cell differentiation may be present. The stroma can be calcified and/or hyalinized.

The immunoprofile is slightly different from its benign and borderline counterparts with loss of p63 positivity but gain of cyclin D1 positivity [17]. They are positive for CK20 unlike TCCs which do not show CK20 staining.

The prognosis is good due to presentation as a low-stage malignancy.

Transitional Cell Carcinomas (TCCs)

Classical TCCs are rare malignancies occurring mostly in the sixth to seventh decades. They usually present as high-stage malignancies. More often, other high-grade and advanced stage carcinomas contain TCC-like areas which can become dominant. This is most often seen in high-grade serous carcinomas, and as such TCCs are now regarded as transitional-like variants of serous carcinomas. Pure TCCs [26] can only be diagnosed when no other background epithelial elements are discernible and the pattern of growth with broad papillary fronds and inverted downgrowths and moderate to marked cytological atypia resembling urinary tract TCCs occupies more than 50 % of the tumor area. They are more common than malignant Brenner tumors and present at an early age than both malignant Brenner tumors and high-grade serous carcinomas. The presenting signs are like other advanced stage ovarian carcinomas and are nonspecific. TCCs are solid and/or cystic with areas of hemorrhage, necrosis, and calcification. In the cystic areas, there can be fronds, excrescences, and nodules. Microscopically, there are inverted papilloma-like features (Fig. 13.7). In some cases, there is a pure solid growth. Diffuse, insular, and trabecular patterns may also be seen. Occasionally there are small acinar spaces in the papillary or solid tumor areas imparting appearances of an endometrioid carcinoma. Squamous metaplasia may be seen. Slit-like gland spaces can be seen and these together with molecular and immunohistochemical data support the notion that they are essentially high-grade serous carcinomas [27]. Invasion into the stroma is seen at the bases of the papillae and is usually haphazard. Focal high-grade nuclear features with the

Table 13.4 The immunohistochemical profile of transitional cell carcinomas

Positive	Negative
CK7	CK20
WT1	Cyclin D1
p16	
p53	
CA125	
CEA (+/–)	
CA 19.9 (+/–)	
p63 (+/–)	

presence of bizarre giant cells can be seen. The cells show severe cytological atypia and often high mitotic activity. Table 13.4 includes the main immunohistochemical profile of transitional cell carcinomas.

It has been known for some time that TCCs are more chemosensitive and have a better prognosis than serous carcinomas, and it may be that TCC-like variants of serous carcinoma represent a better prognostic group as a result of earlier presentation and at a lower stage. Recently a transitional-like morphology in ovarian endometrioid carcinomas has been described [28]. It appears to be distinct from conventional TCCs and high-grade serous carcinoma and does not give rise to a worse prognosis.

Transitional cell tumors can be diagnosed at frozen section, but toward the malignant end of the spectrum, other tumors need consideration. Classical TCC patterns can be recognized, but this is dependent on sampling and it has to be remembered that TCC-like areas are not uncommon in other epithelial malignancies, particularly high-grade serous carcinomas. It could be difficult to differentiate between benign and malignant Brenner tumors when there is no cystic component. One has to rely on identifying cytological and nuclear atypia with a high mitotic count in the malignant tumors.

The differential diagnoses of Brenner tumors and TCCs are mostly limited. There is usually no problem in distinguishing benign from borderline Brenner tumors, the latter being cystic and comprising papillary tumor. Borderline Brenner tumors do not show destructive stromal invasion and therefore can be distinguished from malignant tumors. The latter are also more likely to be solid and cytologically more atypical. TCCs and malignant Brenner tumors are easily distinguished from each other by the absence of benign Brenner elements in the former. If TCCs contain any areas resembling high-grade serous carcinomas, then a diagnosis of serous carcinoma is more appropriate. Ovarian TCCs can represent metastasis from the urinary tract, but the latter are deeply infiltrative and show other characteristics of metastases and are CK20 positive and CK 7 negative. Occasionally, benign and borderline Brenner tumors may be confused with adult granulosa cell tumors because of the nested growth pattern and nuclear grooves in the tumor cells. Benign tumors can also be confused with endometrioid adenofibromas with squamous metaplasia. Malignant tumors can be confused with undifferentiated carcinoma, granulosa cell tumors, serous carcinomas, and endometrioid carcinomas.

Mixed Epithelial Tumors

Mixed tumors are diagnosed when a second component occupies more than 10 % of the tumor area. These are now diagnosed less frequently as recognition of individual subtypes is improved. They can occur in benign tumors but are more common with malignancies. The metaplastic Brenner or mixed Brenner-mucinous tumor (described above) is the best example of a benign mixed epithelial tumor. The most common combined malignancy is endometrioid with clear cell carcinoma. These often exist on a background of endometriosis [29]. Other mixed epithelial malignancies also occur with endometriosis albeit to a lesser degree. Mixed endometrioid and clear cell carcinomas can show a collision tumor pattern (Fig. 13.8) or a well-integrated, intimate admixture pattern. Serous carcinomas can be mixed with high-grade endometrioid carcinomas and/or undifferentiated carcinomas, but generally, these behave like serous carcinomas and are best designated as such. Similarly, clear cell differentiation and even clear cell carcinoma areas can be seen in

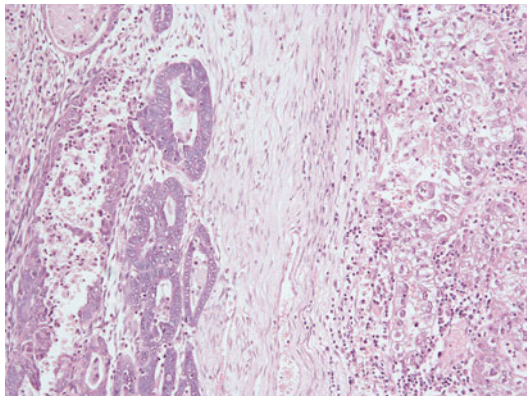

Fig. 13.8 Mixed carcinoma with a collision tumor pattern. Endometrioid carcinoma is on the left and clear cell carcinoma on the right. In other areas, there was an intimate admixture

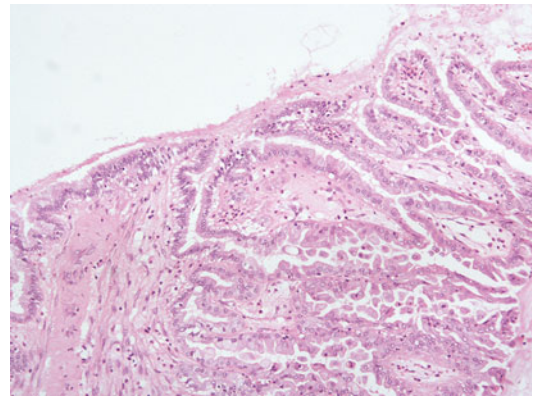

Fig. 13.9 Borderline seromucinous tumor. There are architectural features of a borderline serous tumor but the lining cells are mucinous. Note the typical neutrophil polymorphs in the papillary cores

serous carcinomas, but the prognosis and behavior of such malignancies is like pure high-grade serous carcinoma.

The general rule for mixed tumors is that small areas of a minor other epithelial component can be ignored unless these show high-grade nuclear features or borderline tumor areas in the context of an otherwise benign tumor, in which case it has to be mentioned and will play a role in the overall prognosis.

Among borderline tumors, the most common combination is mucinous with serous, and these are termed borderline seromucinous tumors or borderline Mullerian mucinous or endocervical-like mucinous tumors. In these tumors, there can be other epithelial-type differentiations. It is considered that these behave like borderline serous tumors. They are less common than borderline mucinous tumors of intestinal type and much smaller. They are frequently bilateral and can be multiloculated and have areas of hemorrhage in the wall representing foci of endometriosis which is frequently associated with this subtype of mixed epithelial tumor. Microscopically, the epithelial proliferation is serous in type usually with multiple hierarchical papillary tufts and the cytology is mostly mucinous in nature (Fig. 13.9). Acute inflammatory cells are almost always present (see Chap. 9). TCCs can occur with other carcinomas such as endometrioid and mucinous adenocarcinoma.

Immunohistochemistry is usually not required to differentiate mixed epithelial areas as these usually show classical growth patterns. If classical clear cell carcinomas are present in a true mixed serous and clear cell carcinoma, there is usually no diagnostic problem. However, clear cell differentiation is not uncommon in serous carcinomas, and these areas will show positive staining for ER, WT1, and p53 if they are part of serous carcinomas and negative staining if truly clear cell in nature.

Squamous Cell Tumors [30]

Solitary and pure epidermoid cysts make up around 1 % of ovarian epithelial tumors. They resemble epidermoid cysts in the skin. In the background, there are no teratomatous elements seen, but this can be due to insufficient sampling or could represent overgrowth of a keratinous cyst in a mature teratoma. It could also represent a monodermal teratoma. Interestingly, 50 % of cases with epidermoid cysts have a mature cystic teratoma in the contralateral ovary. As such, these are technically germ cell tumors. Some probably arise from the ovarian surface epithelium or from

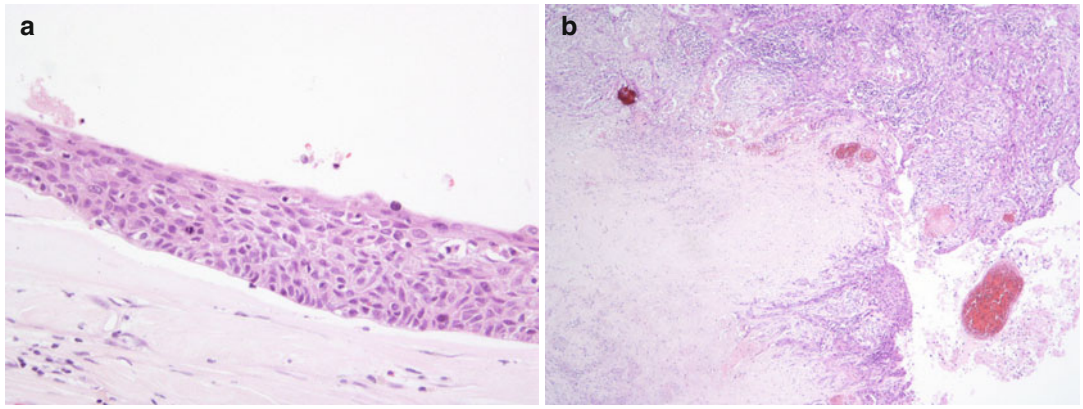

Fig. 13.10 (**a**) In situ squamous carcinoma in a mature cystic teratoma. (**b**) Squamous cell carcinoma arising in a mature cystic teratoma

the rete ovarii. Some epidermoid cysts may be part of a benign Brenner tumor in which there is squamous metaplasia and others represent squamous metaplasia in an endometriotic cyst.

Squamous cell carcinomas can be primary [31], usually representing malignant transformation in an otherwise mature cystic teratoma, and as such is part of germ cell tumors. In these cases, one can sometimes see in situ squamous carcinoma [32] (Fig. 13.10a) and these areas are frequently p16 positive. The invasive component can present as a mural nodule and can breach the ovarian capsule. There are areas of hemorrhage and necrosis apparent. Microscopically, they are moderately to poorly differentiated squamous cell carcinomas (Fig. 13.10b). Primary squamous cell carcinomas are more aggressive than metastatic squamous cell carcinoma to the ovary [33].

Endometrioid adenocarcinomas of the ovaries can display squamous metaplasia, and if the latter is extensive, it can mimic pure squamous cell carcinomas. Squamous cell carcinomas can metastasize to the ovaries usually from the cervix either via the lymphatics or blood vessels or even as contiguous growth through the uterine corpus and tubes. They can also metastasize from other organs. Very rarely, squamous cell carcinomas arise with unknown histogenesis and these are designated squamous cell carcinomas, not otherwise specified.

These tumors do not pose any problems at frozen section and do not require immunohistochemistry for diagnosis.

Other Epithelial Tumors in Mature Teratomas

Similar to squamous tumors, other epithelial tumors can arise secondarily in mature teratomas. These include sebaceous adenomas and carcinomas, adnexal neoplasms, salivary gland tumors, thyroid tumors, and basal cell carcinoma. Teratomatous adenocarcinomas can also be found.

Hepatoid Carcinomas [34]

These are also rare malignancies and resemble hepatocellular carcinoma in the liver. They occur in postmenopausal patients and are at an advanced stage at presentation. The tumor cells are large and eosinophilic and arranged in sheets, cords, or trabeculated (Fig. 13.11). Hyaline bodies and bile may be seen. Immunohistochemically, they are AFP and alpha-1 antitrypsin positive and are alpha-inhibin negative [35]. They may be associated with serous and endometrioid carcinomas. The differential diagnosis is metastasis

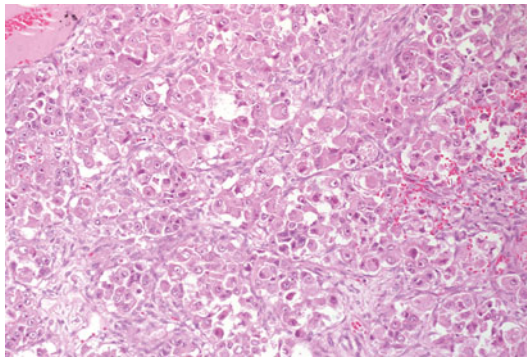

Fig. 13.11 Hepatoid carcinoma. Large eosinophilic cells arranged in a sheet. Hyaline bodies are apparent and there are prominent nucleoli in vesicular, pale nuclei

of hepatocellular carcinoma from the liver, yolk sac tumor with a hepatoid pattern, and steroid cell tumors.

Mixed Epithelial-Mesenchymal Tumors

Adenosarcomas of the ovaries are extremely rare [36, 37] and usually arise in the setting of endometriosis where both epithelial and stromal endometrioid components are present. They are usually unilateral and solid with numerous small cysts. The diagnostic criteria are similar to those in the uterus although one does not see polypoid tumors. The characteristic staghorn glands and condensed stroma around these glands are pathognomonic of this entity. The glandular component is mostly endometrioid and the stromal component is reminiscent of a cellular fibroma or low-grade endometrial stromal sarcoma. Stromal mitotic activity is variable as is the pleomorphism. Sarcomatous overgrowth (Fig. 13.12) may be seen, and in some of these cases, particularly if not adequately sampled, the epithelial component can be overlooked. These tumors appear to behave more aggressively than the uterine counterparts.

Carcinosarcomas (malignant mixed mesodermal tumors) are rare [38] and comprise between 1 and 7 % of ovarian carcinomas depending on which diagnostic criteria are used. They occur in older women and are often large solid/cystic

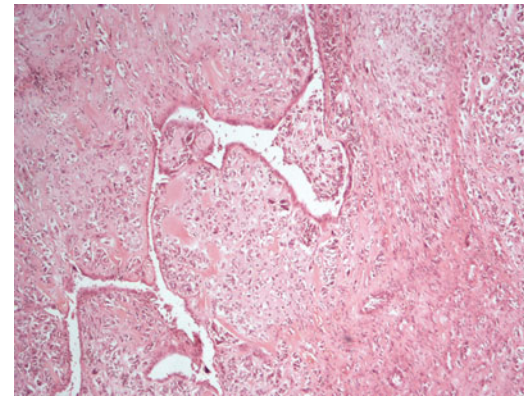

Fig. 13.12 Sarcomatous overgrowth in an adenosarcoma of the ovary. Occasional compressed glands are noted, but there is abundant surrounding malignant mesenchymal tissue

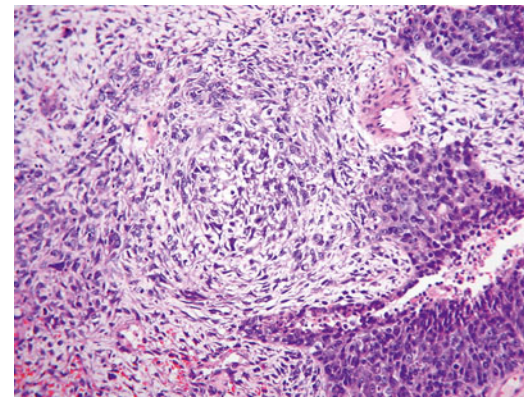

Fig. 13.13 Carcinosarcoma. High-grade carcinoma with sarcoma intimately admixed

tumors up to 20 cm in diameter. In younger women, they occasionally arise in endometriosis. They are bilateral in 30 % of cases. The macroscopic appearances are often similar to high-grade serous carcinomas as is the stage at presentation. Microscopically, there is an intimate and complex admixture of carcinomatous with sarcomatous elements (Fig. 13.13). The carcinoma component is usually high-grade and either serous or endometrioid or undifferentiated in subtype. If there is mucinous carcinoma, care has to be taken not to overlook sarcomatous mural nodules in otherwise benign mucinous tumors. The sarcomatous component is homologous in 30 % of cases as in the uterine carcinosarcomas. Heterologous sarcoma is sometimes found and

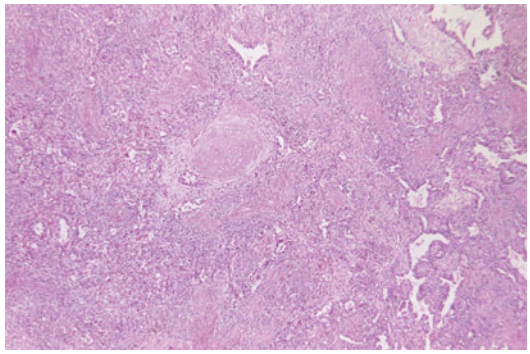

Fig. 13.14 Carcinosarcoma, heterologous. High-grade carcinoma and sarcoma with a focus of chondrosarcoma. The inset shows a high-power view of another focus of chondrosarcoma of the same case

can comprise chondrosarcoma (Fig. 13.14), rhabdomyosarcoma, osteosarcoma, or liposarcoma singly or in combination. The sarcomatous component can be minimal and may be overlooked if not adequately sampled. Equally, it can overgrow the carcinomatous areas rendering a diagnosis of undifferentiated high-grade sarcoma if an epithelial component is not sought.

In immunohistochemical and molecular studies, it has been shown that the carcinosarcomas are in fact monoclonal and hence undifferentiated or metaplastic carcinomas with sarcomas developing from the high-grade carcinomas. This is similar to uterine carcinosarcomas. Sarcomatous areas may stain positively with epithelial markers. The only other immunostains that may be required are those to confirm the presence of rhabdomyosarcomatous differentiation (desmin, myo D1, myogenin).

The clinical course is considered to be similar to serous carcinomas, and very often in advanced age malignancies, it is the carcinomatous component that spreads outside of the ovaries and in fact may mislead if the initial diagnosis is made by a small peritoneal/omental biopsy that has picked up only the carcinomatous component. The overall treatment strategy and outcomes are similar to high-grade serous carcinoma and exact classification may not be so relevant. However, a recent study has shown a worse prognosis of carcinosarcomas in comparison with serous carcinoma [39]. In cases where the sarcomatous component also spreads outside of the ovary, the prognosis is definitely worse [40].

If carcinosarcomas are received for frozen sections, it is quite possible that only one or the other component is recognized, but either tends to be high-grade, and for the purpose of frozen section diagnosis, that is all that one requires. It might be prudent to sample more blocks of the tumor if the initial one shows sarcomatous tissue. Conversely, the presence of sarcoma may not be considered if high-grade carcinoma is already recognized in the initial blocks.

The main differential diagnoses, including at frozen section, are high-grade endometrioid adenocarcinomas with spindle cell solid growth, immature teratomas, poorly differentiated Sertoli-Leydig cell tumors, dedifferentiated endometrioid adenocarcinomas where the undifferentiated component might be dominant, endometrial stromal sarcomas with sex cord-like differentiation, and combined adenocarcinoma with neuroendocrine carcinoma elements including small cell carcinomas. Carcinosarcomas may arise as malignant transformation in mature teratomas [41].

Tumors of the Rete Ovarii

Occasionally, tumors arise in the rete ovarii and most of these are benign cysts or cystadenomas lined by bland, attenuated, or cuboidal cells without cilia. They occur in a wide age range and present with a mass effect and occasionally with hormonal manifestations. They may be confused with serous cystadenomas of the ovary, and the main distinguishing feature is the lack of ovarian stroma in the cyst wall of a rete lesion. Instead, there is smooth muscle. Solid adenomas and adenomatous hyperplasia can also occur, the former being circumscribed. Adenocarcinomas are rare and can be tubular or papillary or even solid with a variable degree of atypia and mitotic activity. Walthard's rests and Brenner tumors can arise in the rete ovarii as a result of transitional cell differentiation. These lesions stain positively with CA125, CD10, PR, CAM5.2, and EMA. Apart from serous cystadenomas,

the other main differential diagnosis is adnexal tumor of probable Wolffian origin (FATWO) which can be confused with adenomas of the rete ovarii. FATWOs are inhibin and vimentin positive and EMA is negative.

Learning Points

- Undifferentiated carcinomas are rare. Every effort must be made to identify areas of the more common high-grade carcinomas, particularly serous carcinomas.
- Small cell carcinoma, hypercalcemia type, is the most common undifferentiated carcinoma and is characterized by small cells with nuclear molding arranged in sheets within which follicle-like spaces are present.
- Most Brenner (transitional cell) tumors are benign. Borderline Brenner tumors are rare and characterized by papillary proliferation of transitional cells.
- Transitional cell carcinomas are more common than malignant Brenner tumors but rare as pure malignancies. They do not contain benign Brenner elements in the background and are likely to represent transitional-like variants of high-grade serous carcinomas.
- True mixed epithelial carcinomas are also rare with the majority composed of variants of high-grade serous carcinoma. They are only diagnosed when genuine; characteristic features of the minority component occupying more than 10 % area of the tumor are seen. The most common is endometrioid with clear cell carcinoma which occurs on a background of endometriosis.
- Primary squamous cell carcinomas of the ovary are rare and usually represent malignant transformation in a mature teratoma. Metastases from other sites, particularly the cervix, need to be excluded especially when teratomatous elements are not found. Thorough sampling is essential.
- Carcinosarcomas are rare ovarian malignancies and represent metaplastic carcinomas. The epithelial components are usually high-grade serous or endometrioid carcinomas and the sarcomatous components can be homologous or heterologous.
- For all the rare entities such as undifferentiated carcinomas, squamous and mixed carcinomas, transitional cell carcinomas, and carcinosarcomas, thorough sampling, including at frozen sections, is imperative.
- Immunohistochemistry may be helpful in distinguishing some of these rare entities from the more common ovarian malignancies.

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Germ Cell Tumors of the Ovary and Dysgenetic Gonads

14

Melanie Joy Newbould

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].

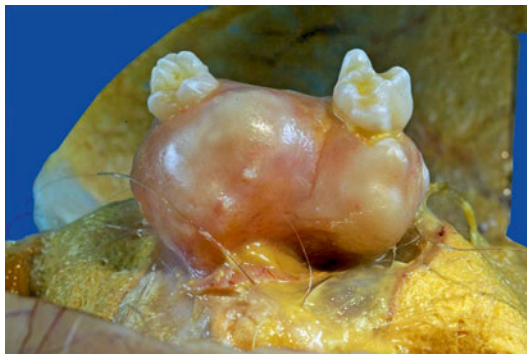

Fig. 14.1 Mature cystic teratoma, opened to show lining and the presence of teeth (Photo provided by Dr. Helen Stringfellow)

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

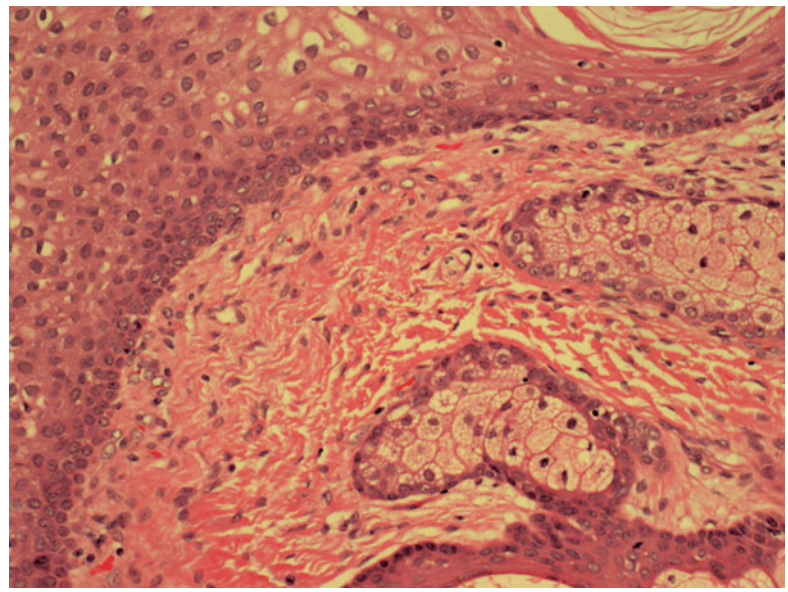

Fig. 14.2 Mature cystic teratoma – epidermal appendages, H&E ×200

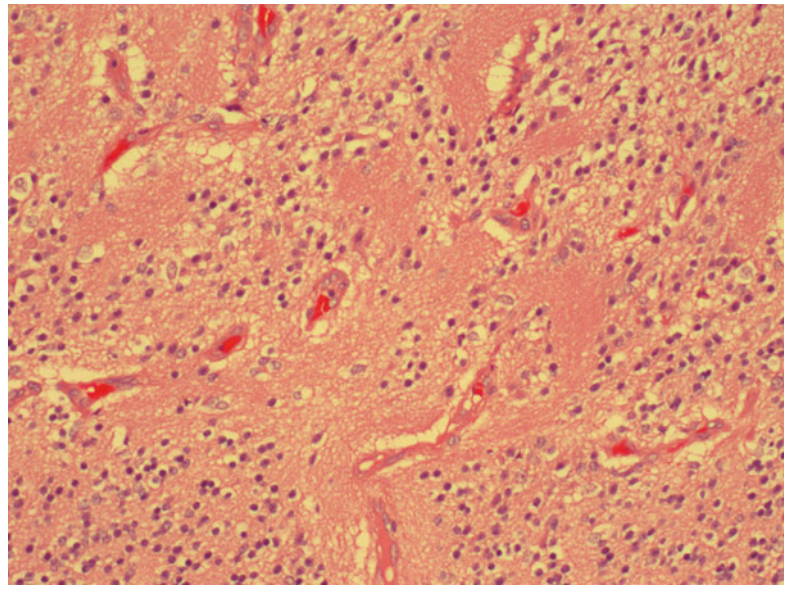

Fig. 14.3 Mature cystic teratoma – glial tissue, H&E ×200

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.

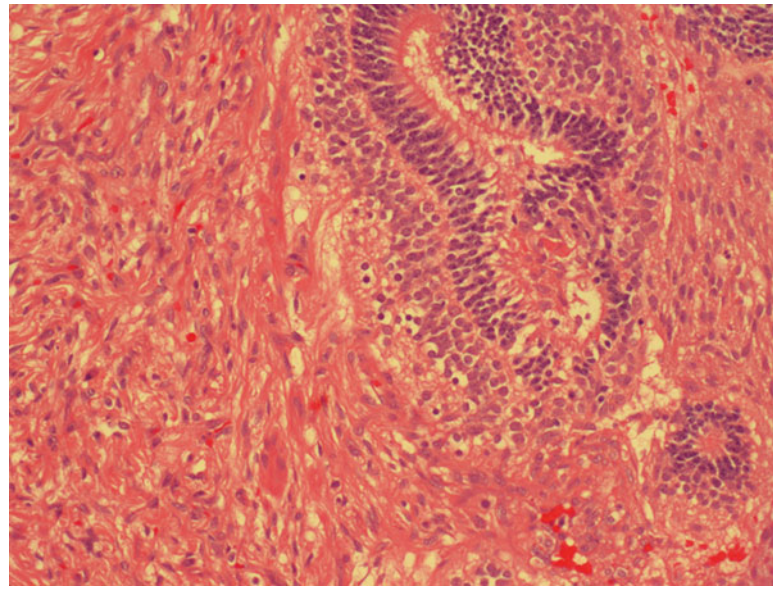

Fig. 14.4 Mature cystic teratoma – microscopic focus of immature neuroepithelium – H&E ×200

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].

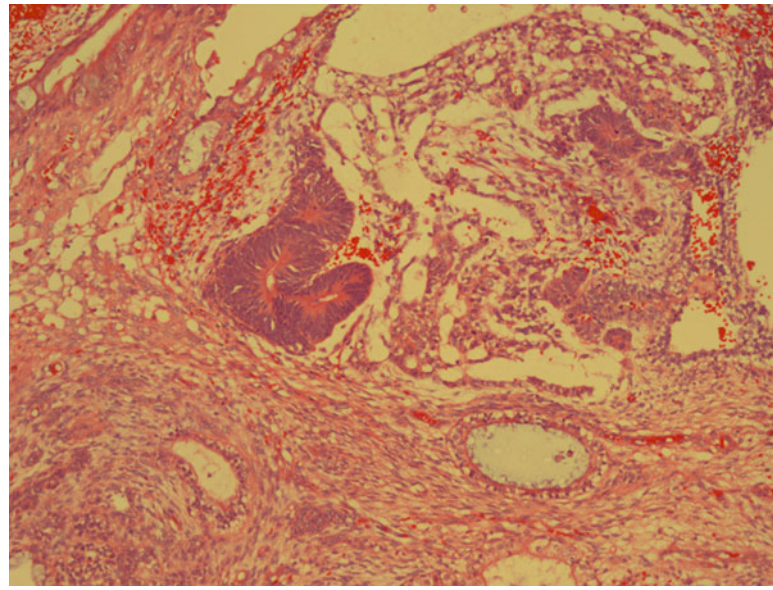

Fig. 14.5 Immature teratoma. Immature mesenchymal and endodermal elements, including a focus of immature neuroepithelium – H&E ×100

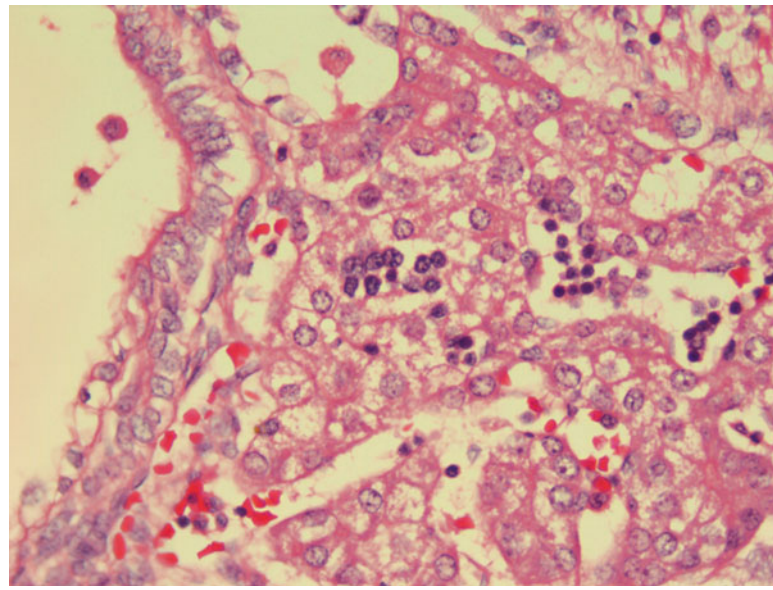

Fig. 14.6 Immature teratoma. Area of hepatic differentiation. Hemopoiesis also present. H&E ×400

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:

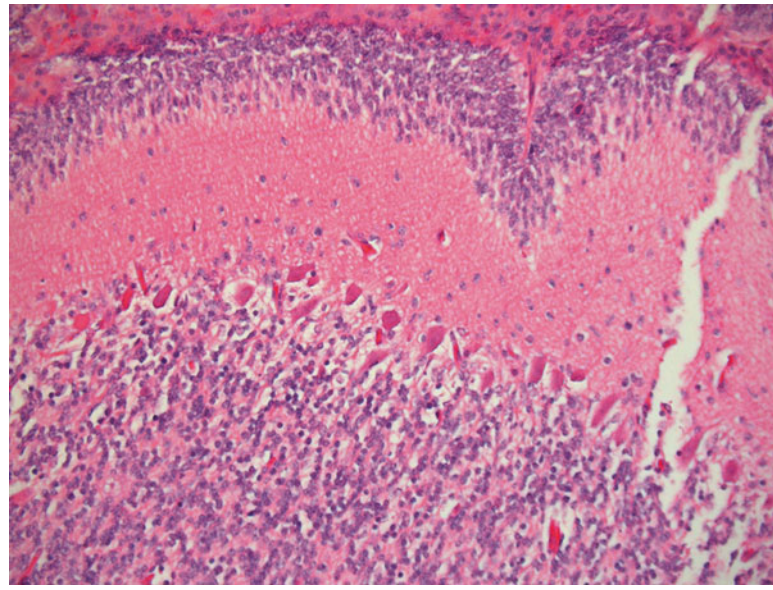

Fig. 14.7 Immature teratoma – cerebellar cortex (of infantile type) – this is not embryonal tissue, but differentiated tissue. The small granular cells may be deceptive, but do not show the mitoses and apoptosis H&E ×200

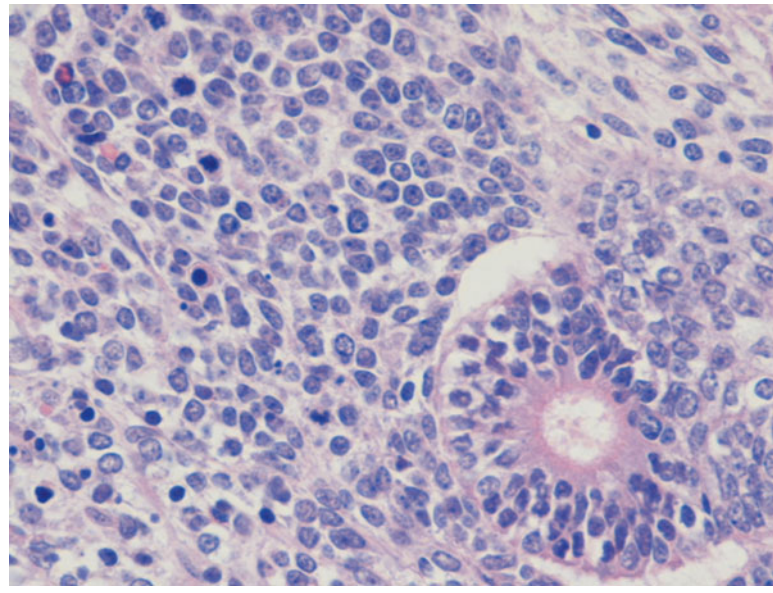

Fig. 14.8 Immature neuroepithelium in an immature teratoma. H&E ×400 (Photo provided by Dr Helen Stringfellow)

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.

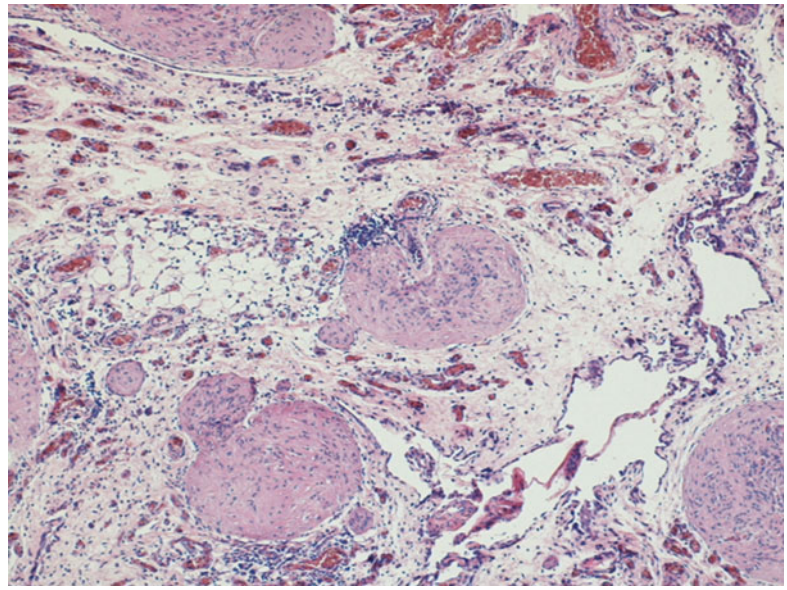

Fig. 14.9 Peritoneal glial nodule – gliomatosis. H&E ×100 (Photo provided by Dr Helen Stringfellow)

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

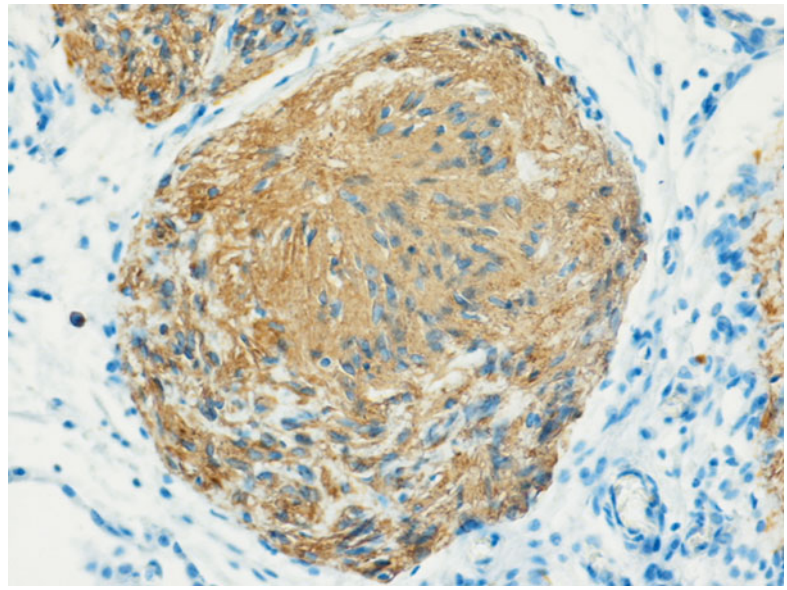

Fig. 14.10 Peritoneal glial nodule – gliomatosis. GFAP ×200 (Photo provided by Dr Helen Stringfellow)

(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

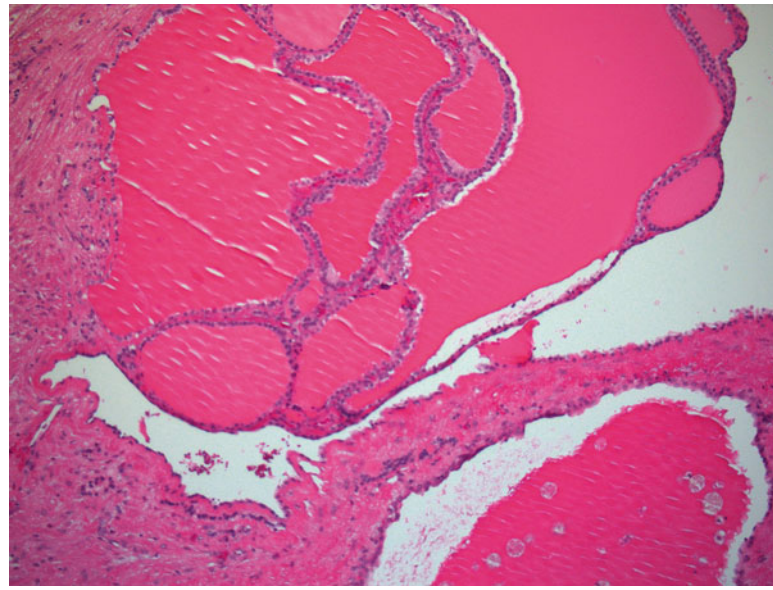

Fig. 14.11 Struma ovarii H&E ×100

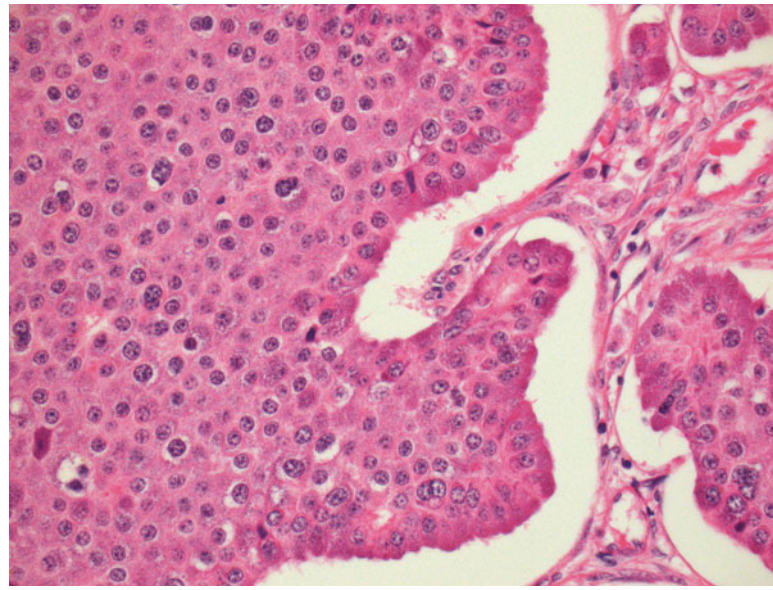

Fig. 14.12 Ovarian carcinoid with an insular pattern. H&E ×200

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

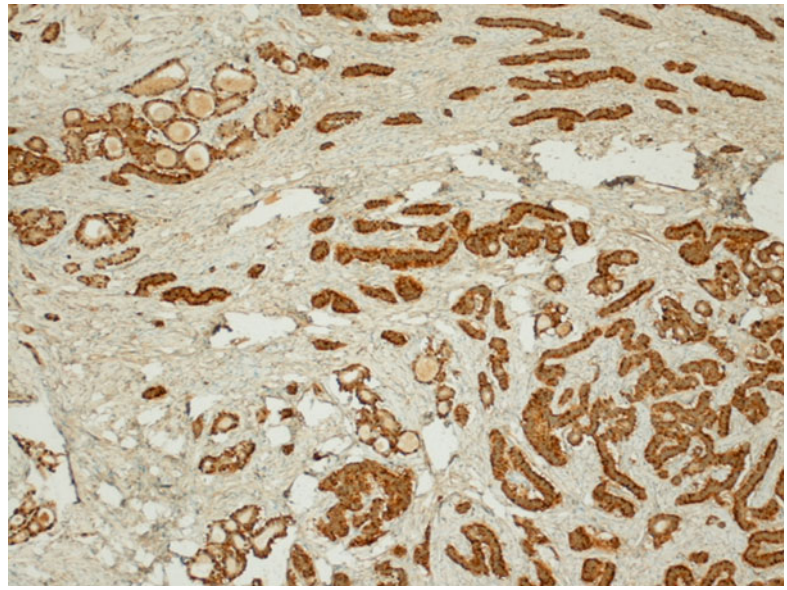

Fig. 14.13 Ovarian carcinoid with a partly trabecular pattern. Chromogranin ×100 (Photo provided by Dr Helen Stringfellow)

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].

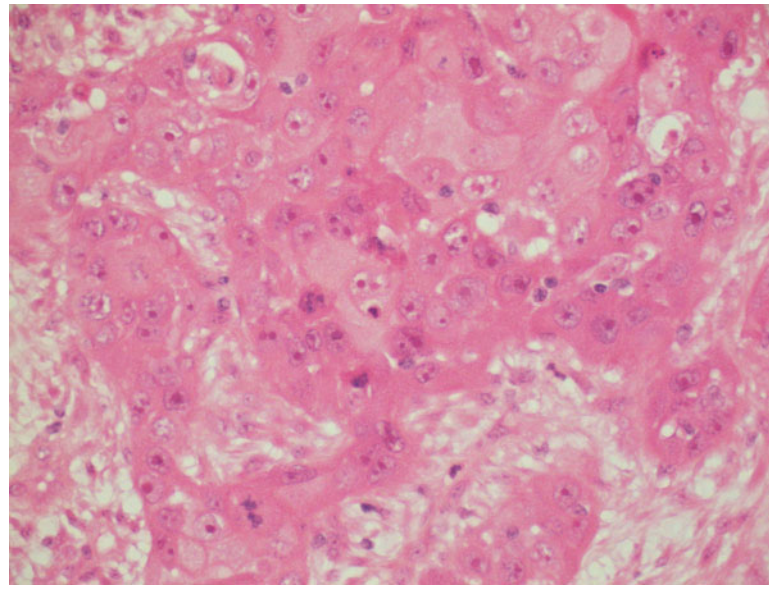

Fig. 14.14 Ovarian squamous carcinoma developing in association with a mature cystic teratoma. H&E ×200

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.

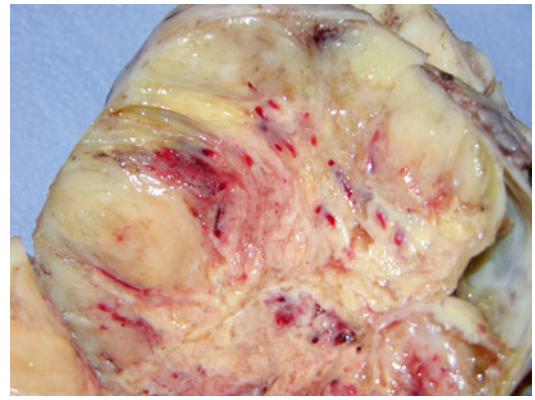

Fig. 14.15 Ovarian dysgerminoma, macroscopic appearance (Photo supplied by Dr Helen Stringfellow)

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

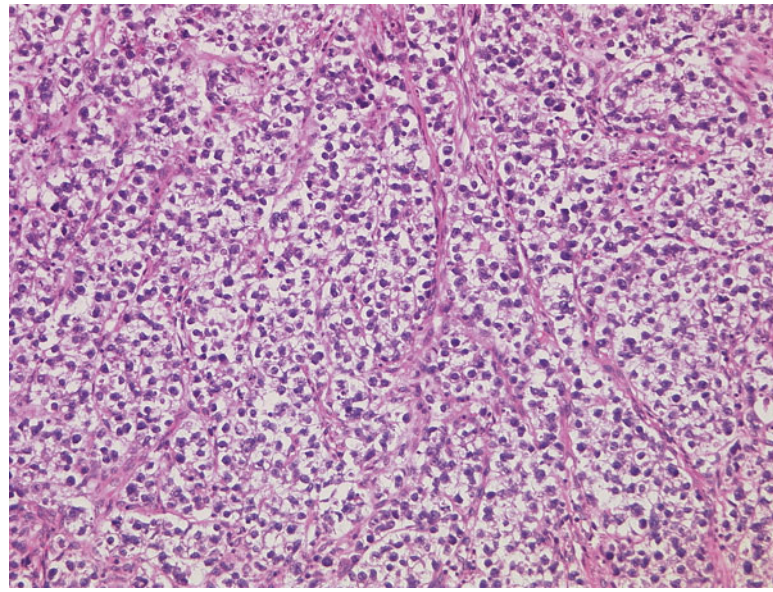

Fig. 14.16 Dysgerminoma. Cells separated by fine collagen bands. H&E ×100 (Photo provided by Dr Nafisa Wilkinson)

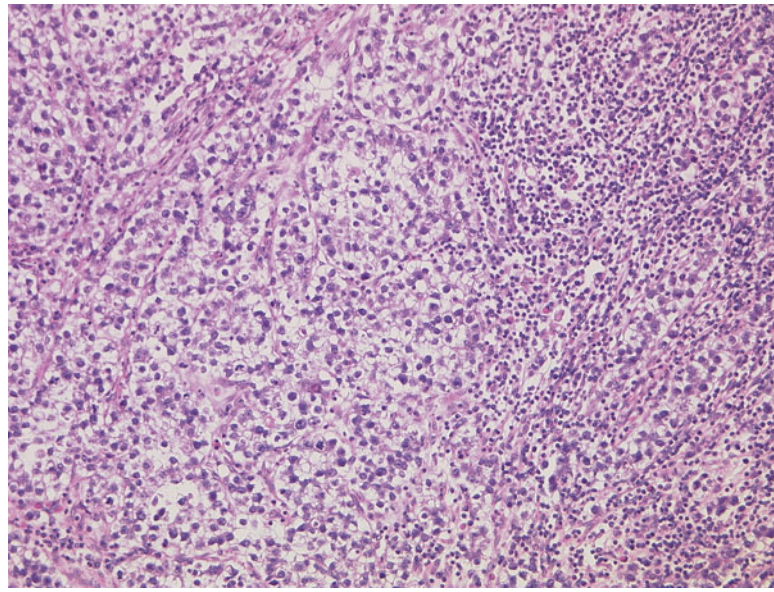

Fig. 14.17 Dysgerminoma with typical lymphocytic aggregates H&E ×100 (Photo provided by Dr Nafisa Wilkinson)

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

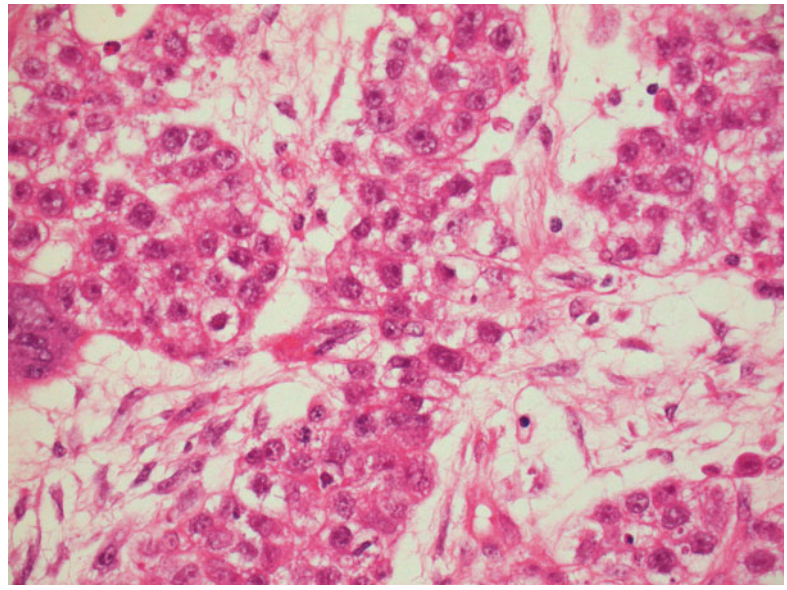

Fig. 14.18 Dysgerminoma – no lymphocytes – differential diagnosis is with other more malignant germ cell tumor types, yolk-sac tumor and embryonal carcinoma H&E ×200

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

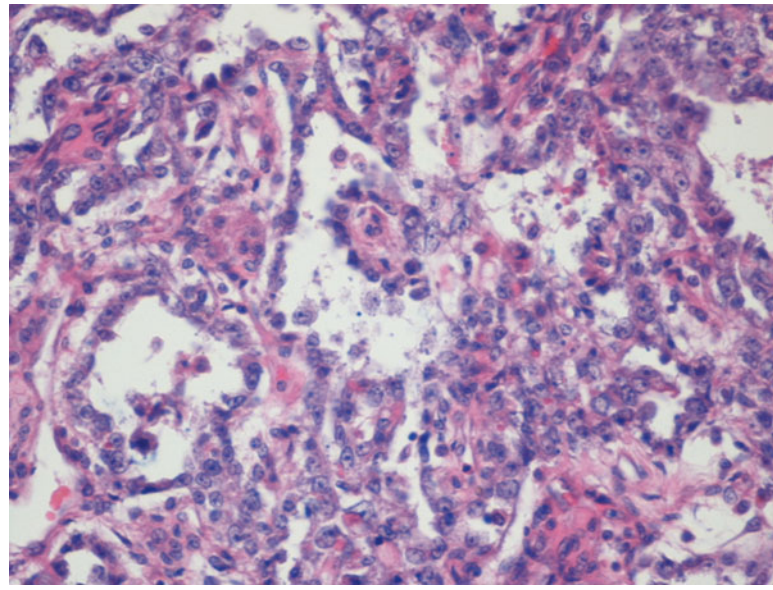

Fig. 14.19 Yolk-sac tumor. Schiller-Duval sinus. H&E ×200 (Photo supplied by Dr Helen Stringfellow)

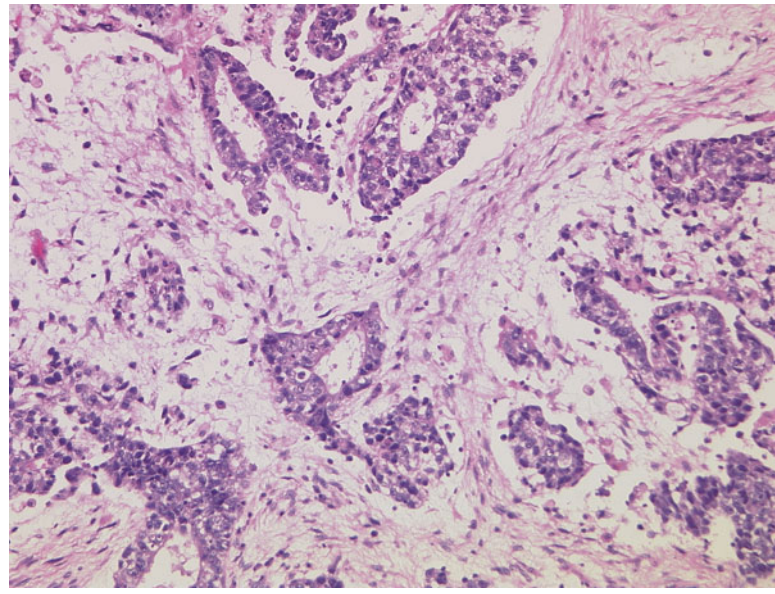

Fig. 14.20 Yolk-sac tumor. Glandular area resembling carcinoma. H&E ×100 (Photo provided by Dr Nafisa Wilkinson)

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].

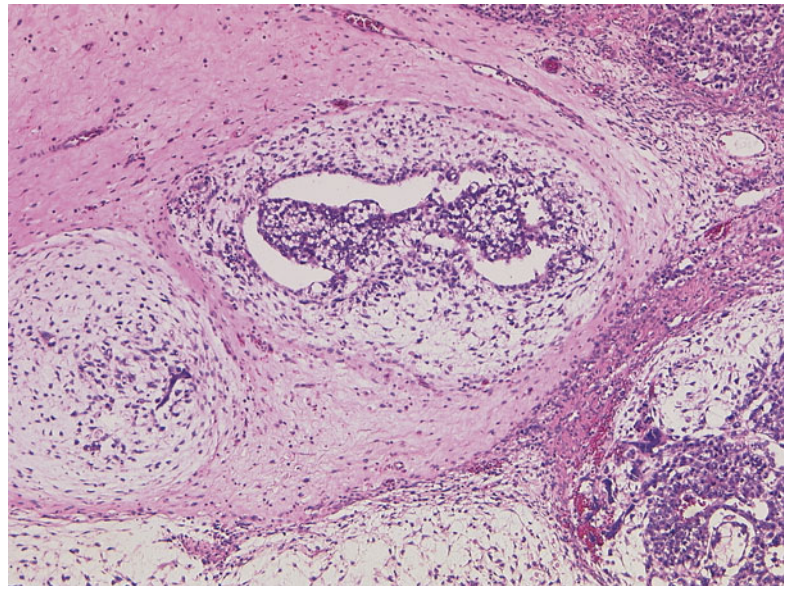

Fig. 14.21 Yolk-sac tumor – area with abundant mesenchyme and only a few islands of primitive epithelium. H&E ×2,100

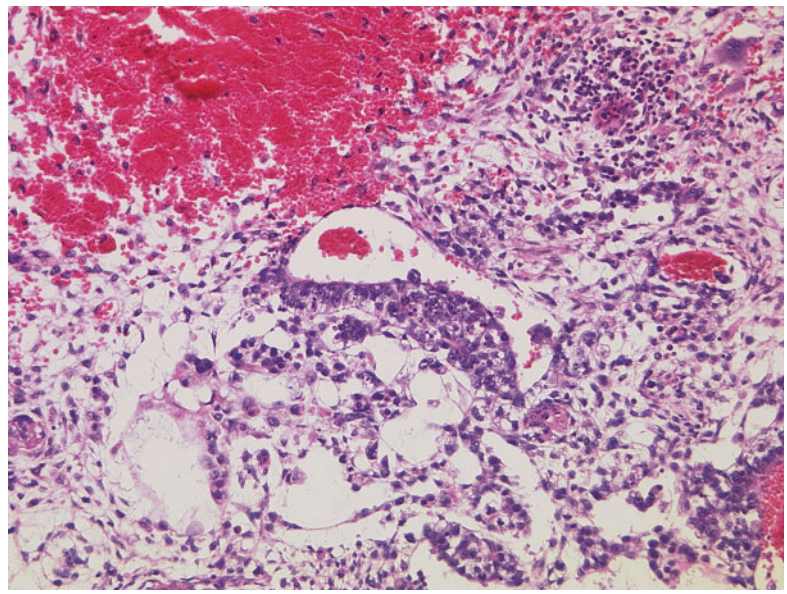

Fig. 14.22 Yolk-sac tumor – hemorrhagic area H&E ×200

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

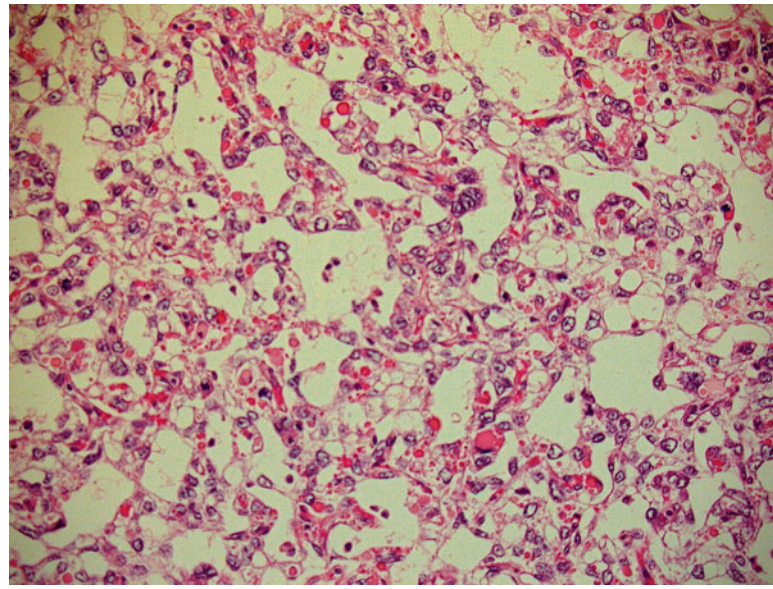

Fig. 14.23 Yolk-sac tumor. Hyaline globules. Here photographed in H&E stain. ×200

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

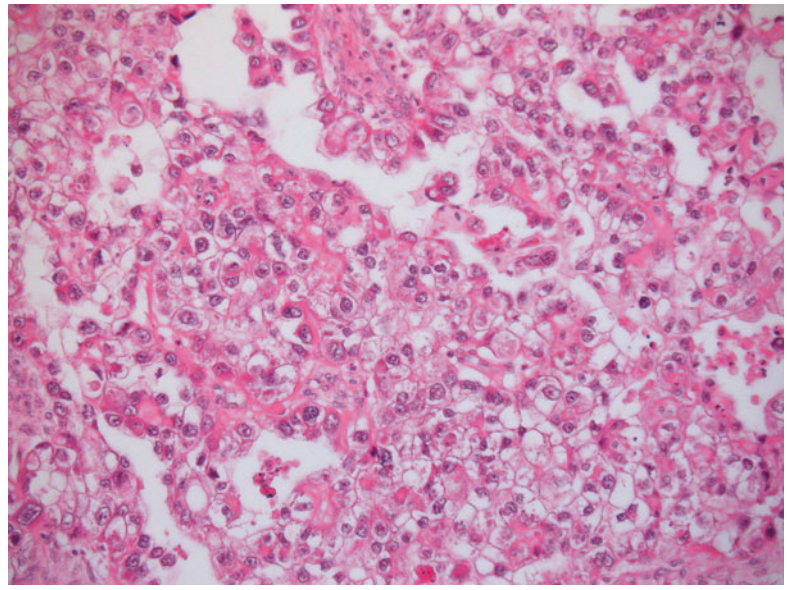

Fig. 14.24 Ovarian clear cell carcinoma H&E ×200

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

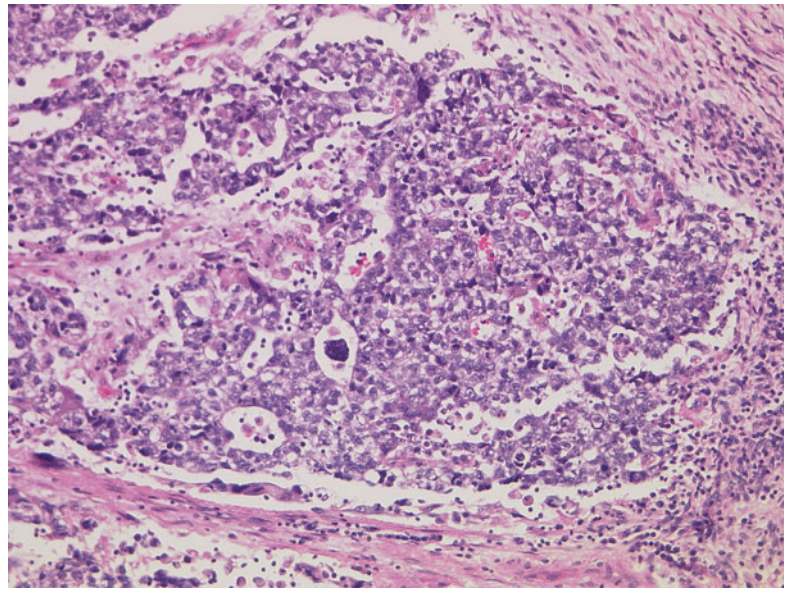

Fig. 14.25 Ovarian embryonal carcinoma H&E ×200

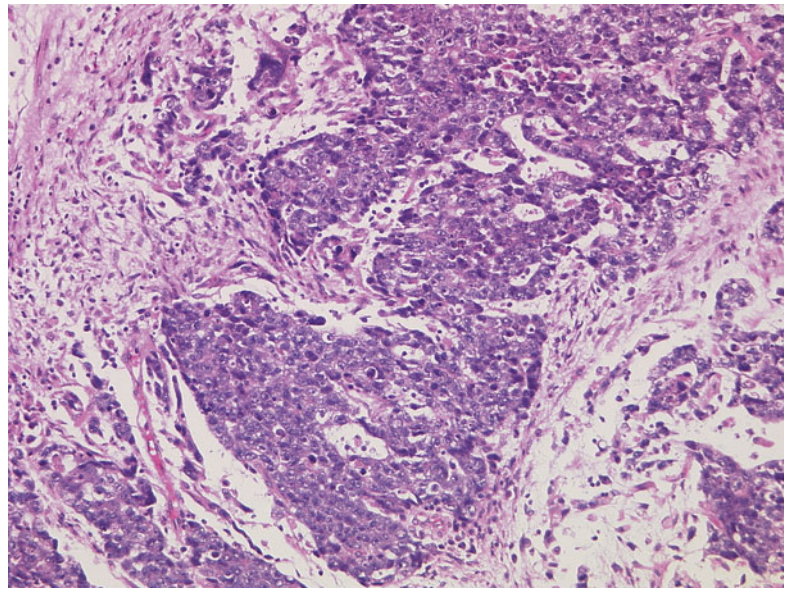

Fig. 14.26 Ovarian embryonal carcinoma H&E ×200

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

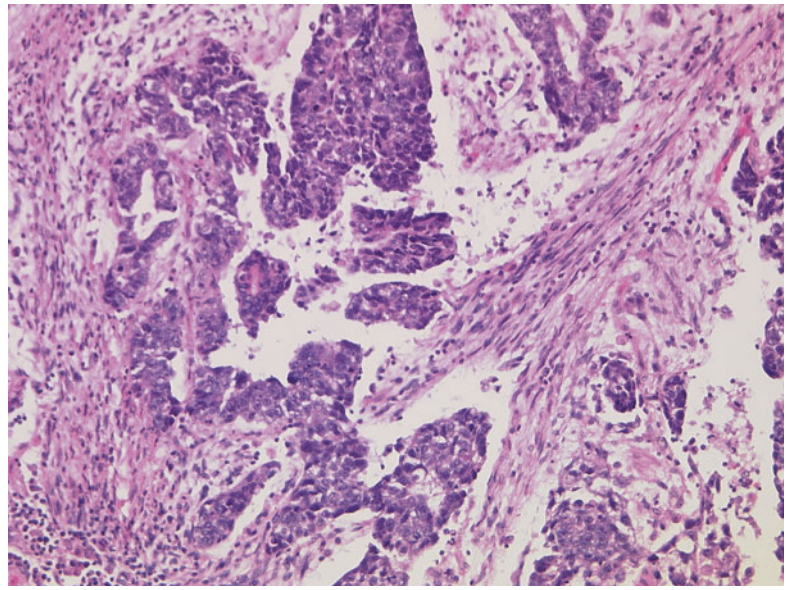

Fig. 14.27 Ovarian embryonal carcinoma H&E ×200

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

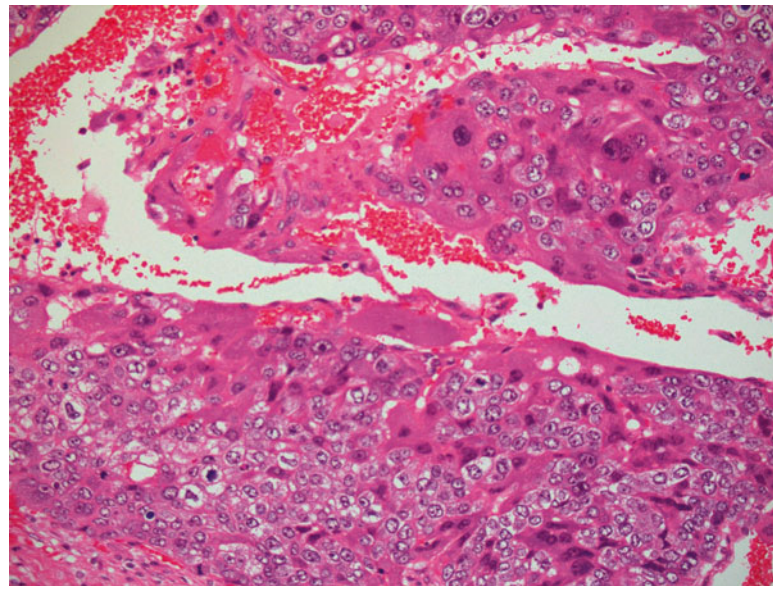

Fig. 14.28 Choriocarcinoma H&E ×400

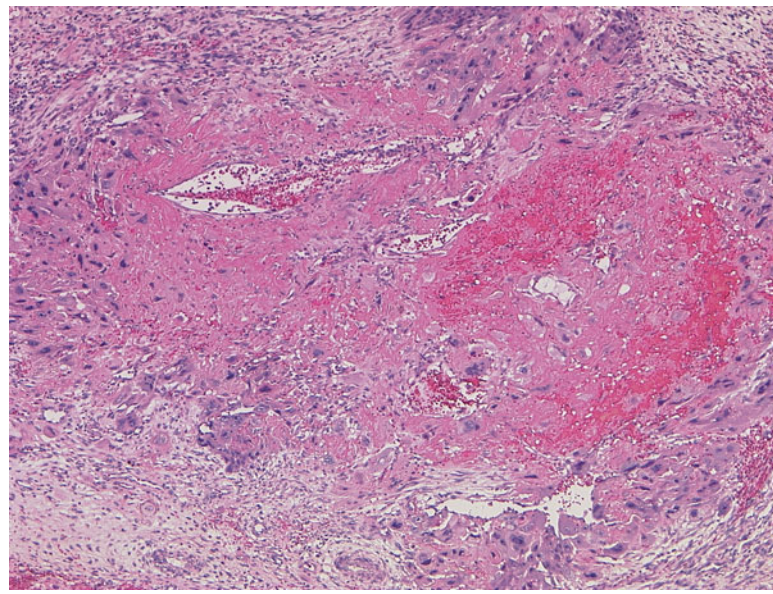

Fig. 14.29 Choriocarcinoma H&E ×200

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

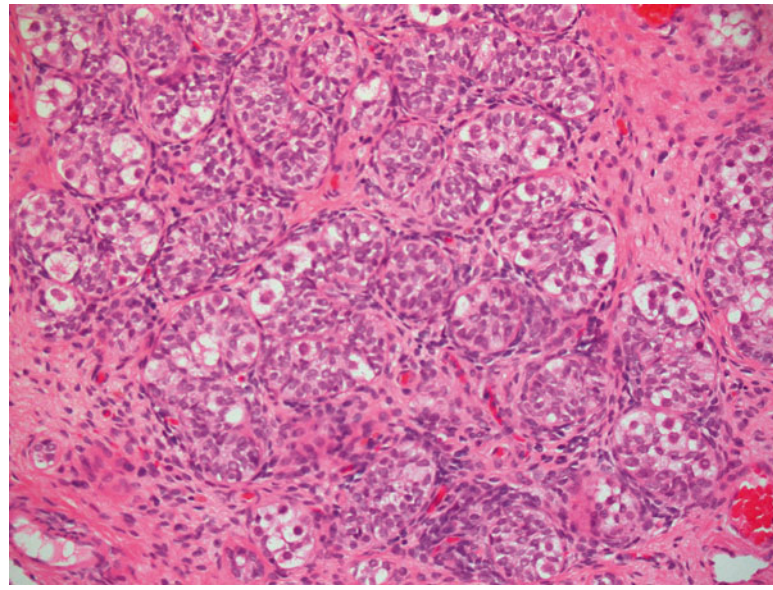

Fig. 14.30 Testis in an individual with 45,X/46XY karyotype. Note prominent immature atypical germ cells, very difficult to distinguish between immaturity and ITGCNU in this situation H&E ×200

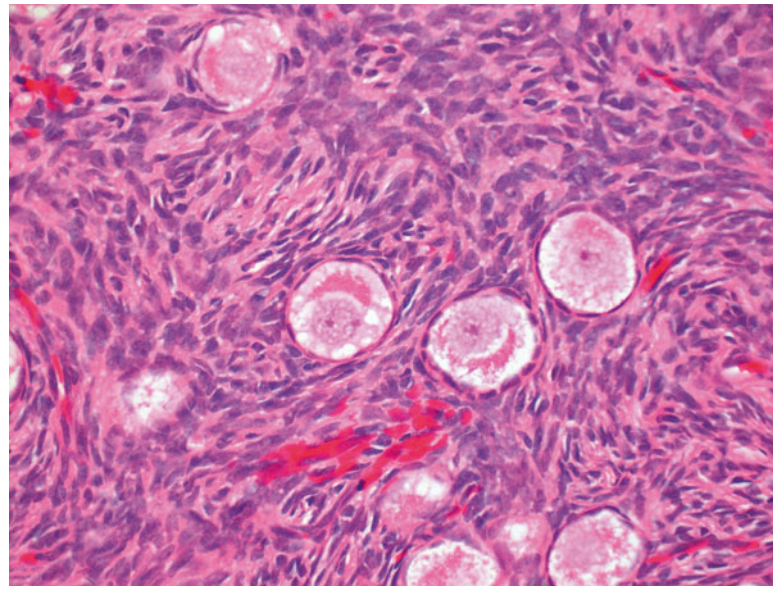

Fig. 14.31 Ovarian tissue in 46,XX individual who also had testicular tissue in the other gonad H&E ×200

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

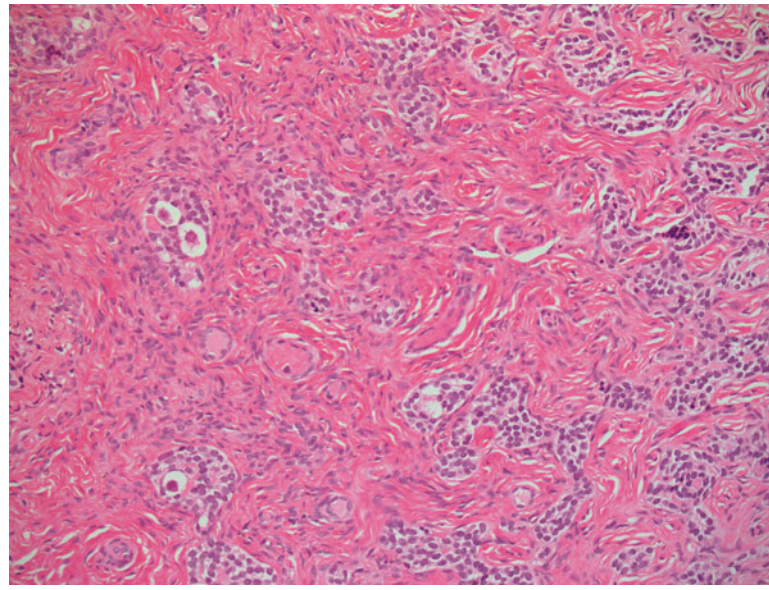

Fig. 14.32 Undifferentiated gonadal tissue, from 46X/46XY individual. Sex cords are not obviously forming seminiferous tubules or follicular structures. Germ cells are present. The morphology here seems suggestive of an early gonadoblastoma. H&E ×200

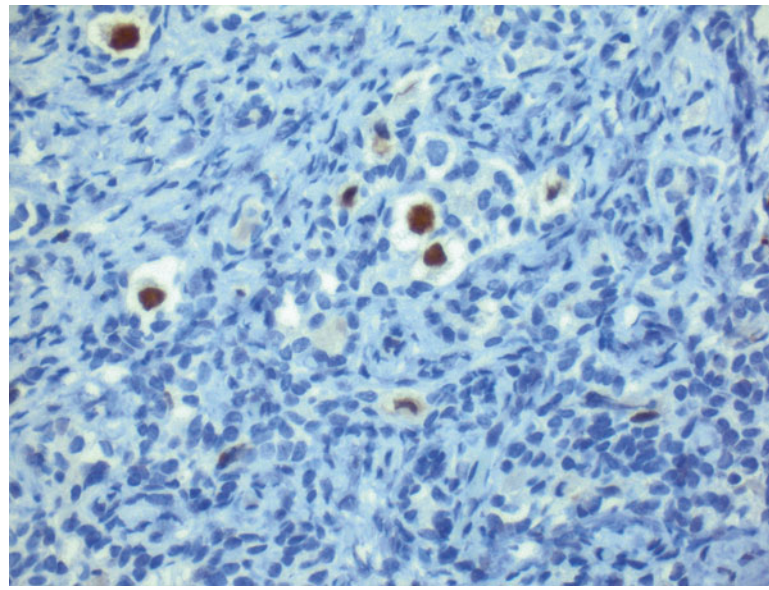

Fig. 14.33 Undifferentiated gonad stained with antibody to OCT3/4. Germ cell nuclei positive ×400

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].

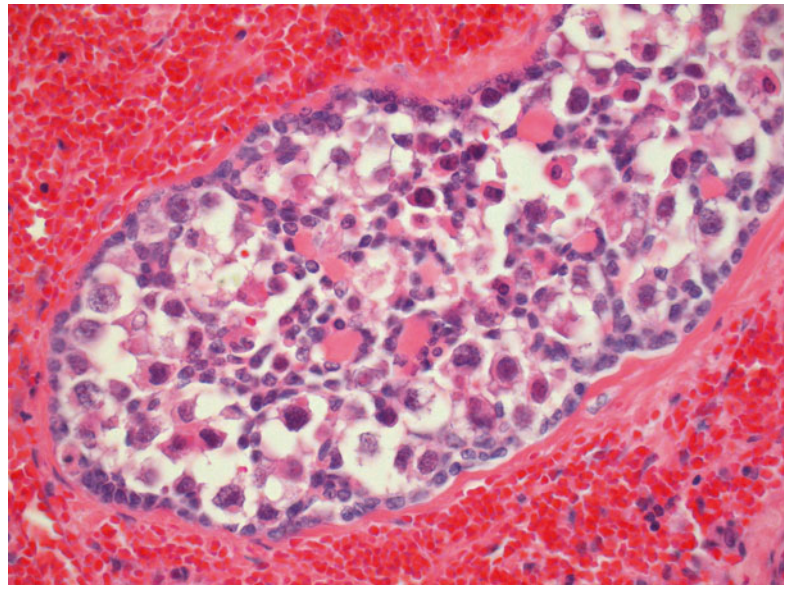

Fig. 14.34 Gonadoblastoma H&E ×200

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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Sex Cord–Stromal Tumors

15

Raji Ganesan

Abstract

Sex cord–stromal tumors of the ovary are believed to arise from and/or to contain combinations of the sex cord and stromal components of the developing gonad. During embryogenesis, condensations of subcoelomic epithelium develop into ovarian cortical stroma, granulosa, theca, Sertoli, and Leydig cells in the female. These tumors comprise about 7 % of all ovarian malignancies. The third WHO classification divides the sex cord–stromal tumors into four groups: granulosa–stromal cell tumors, Sertoli–stromal cell tumors, tumors of mixed or unclassified cell type, and steroid cell tumors. This differs from the earlier WHO classifications by grouping three unusual tumors that show neither preponderant ovarian nor testicular cell type. A brief, modified version of the 2003 WHO classification is presented in Table 15.1.

Introduction

Sex cord–stromal tumors of the ovary are believed to arise from and/or to contain combinations of the sex cord and stromal components of the developing gonad. During embryogenesis, condensations of subcoelomic epithelium develop into ovarian cortical stroma, granulosa, theca, Sertoli, and Leydig cells in the female. These tumors comprise about 7 % of all ovarian malignancies [1]. The third WHO classification [2] divides the sex cord–stromal tumors into four groups: granulosa–stromal cell tumors, Sertoli–stromal cell tumors, tumors of mixed or unclassified cell type, and steroid cell tumors. This differs from the earlier WHO classifications [3, 4] by grouping three unusual tumors that show neither preponderant ovarian or testicular cell type. A brief, modified version of the 2003 WHO classification is presented in Table 15.1.

This chapter aims to be comprehensive and includes all tumor types while concentrating on the more common entities and providing a morphological approach to diagnosis.

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Table 15.1 Classification of sex cord–stromal tumors

1. Sex cord–stromal tumors
(a) Granulosa cell tumors – adult and juvenile
(b) Thecoma–fibroma group including thecoma, fibroma, cellular fibroma
2. Sertoli–stromal tumors
(a) Sertoli–Leydig cell tumor – well, intermediate, poorly differentiated, and retiform variants
(b) Sertoli cell tumor
(c) Stromal–Leydig cell tumor
3. Sex cord–stromal tumors of mixed or unclassified type including sex cord tumors with annular tubules and gynandroblastoma
4. Steroid cell tumors including Leydig cell tumor and steroid cell tumor

Abbreviated and modified from WHO classification 2003

Granulosa–Stromal Cell Tumors

These are tumors that show ovarian-type differentiation. They are subdivided into two large categories, the granulosa tumor group or the thecoma–fibroma group, depending on the presence or absence of granulosa cells. By definition, a neoplasm must contain a granulosa cell component of at least 10 % on any one slide to be diagnosed as granulosa cell tumor [5].

Granulosa Cell Tumors

Epidemiology and Clinical Correlates

Granulosa cell tumors (GCTs) are the most common of the malignant sex cord–stromal tumors of the ovary. Two subtypes of GCT have been described – the juvenile and the adult form, of which the latter is much more common accounting for 95 % of these tumors. These are referred to as adult GCT (AGCT). They occur mostly in perimenopausal women but can occur at any age group [6, 7]. The remaining 5 % of these tumors occur in prepubertal girls and compose most of the juvenile granulosa cell tumor (JGCT) subset. The distinction between the two subsets is not purely age based but also morphological and mutation based. They are likely to represent two different disease processes. Granulosa cell tumors frequently secrete estrogens. This feature causes isosexual pseudoprecocity in JGCTs. In peri- and postmenopausal women, the hyperestrinism can lead to endometrial hyperplasia and even endometrial carcinoma [8]. Rarely, AGCT can be associated with androgenic manifestations, especially when they are cystic [9]. Typically, GCTs are confined to the ovary at the time of presentation – FIGO Stage IA. They are very vascular and can present as gynecological emergencies due to spontaneous rupture [10]. AGCTs are typically indolent tumors with tendency for late recurrences, the longest noted interval to recurrence being 37 years [11]. Tumor recurrence or metastasis is rare in JGCTs, and if this neoplasm recurs, this is usually early [12]. The literature is conflicting with regard to prognostic parameters. The strongest proven link is with higher stage at diagnosis and outcome. Other parameters inconsistently reported as being of prognostic importance include patient age, tumor rupture, tumor size, mitotic index, and nuclear atypia [13, 14].

Macroscopy

AGCTs

Most AGCTs are unilateral and average about 12 cm in maximum dimension. Typically, they have a variably hemorrhagic, tan to yellow, solid or solid–cystic cut surface. Rare tumors may be completely cystic [5, 15].

JGCTs

Most neoplasms have a solid and cystic cut surface with unilocular or multilocular cysts [12, 16, 17]. Solid foci are tan to yellow in color with occasional areas of necrosis and hemorrhage.

Microscopy

AGCTs

The characteristic histological feature of AGCTs is a monomorphic proliferation of uniform cells with scanty cytoplasm. Typically, AGCT nuclei have a longitudinal groove (Fig. 15.1). A wide variety of growth patterns are seen, generally in combination. Diffuse sheets are most common with microfollicular (Fig. 15.2), macrofollicular, insular, trabecular, watered-silk (Fig. 15.3), gyriform, and tubular arrangements. The characteristic feature of microfollicular tumors is the

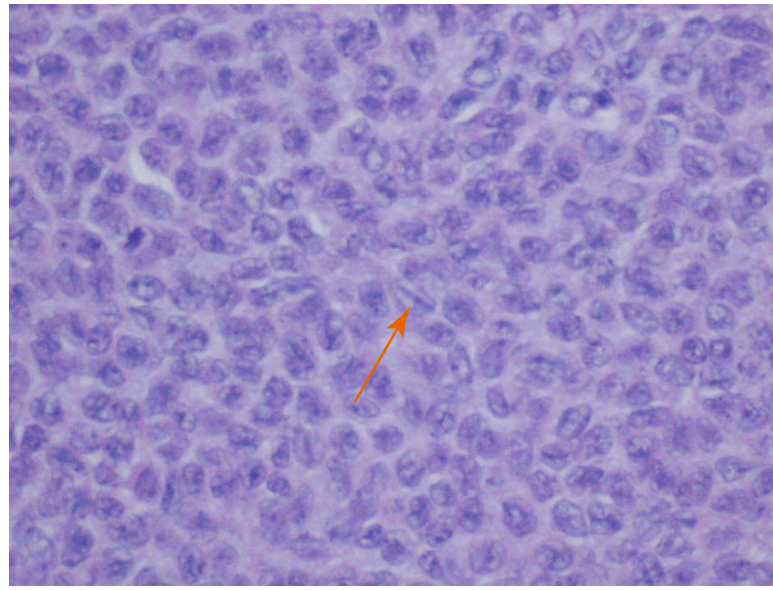

Fig. 15.1 Longitudinal nuclear grooves (*arrowed*) are typical of adult granulosa cell tumors (H&E ×400 magnification)

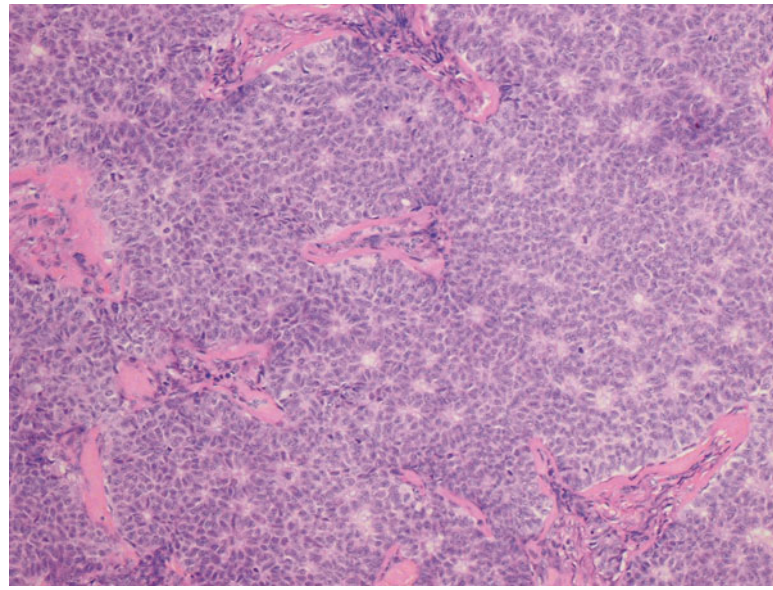

Fig. 15.2 In the microfollicular pattern of adult granulosa cell tumors, there is a central follicle-like space surrounded by haphazardly oriented granulosa cells (H&E. ×100 magnification)

formation of Call–Exner bodies which is best described as a small round space containing eosinophilic material and surrounded by haphazardly oriented granulosa cells with longitudinal nuclear grooves. Mitotic activity is usually brisk (Fig. 15.4). Infrequently, bizarre nuclei can be seen and their presence does not alter diagnosis or prognosis [18]. In about 2 % of cases, the tumor cells contain abundant eosinophilic cytoplasm, round to oval nuclei with conspicuous nucleoli, constituting the luteinized variant of AGCT [19, 20] (Fig. 15.5). Granulosa cell tumors, both adult and juvenile variants, may have a pseudopapillary pattern (Fig. 15.6). In contrast to true papillae that have stromal cores of fibrovascular or edematous connective tissue, pseudopapillae develop as a secondary or degenerative phenomenon and lack true stromal cores

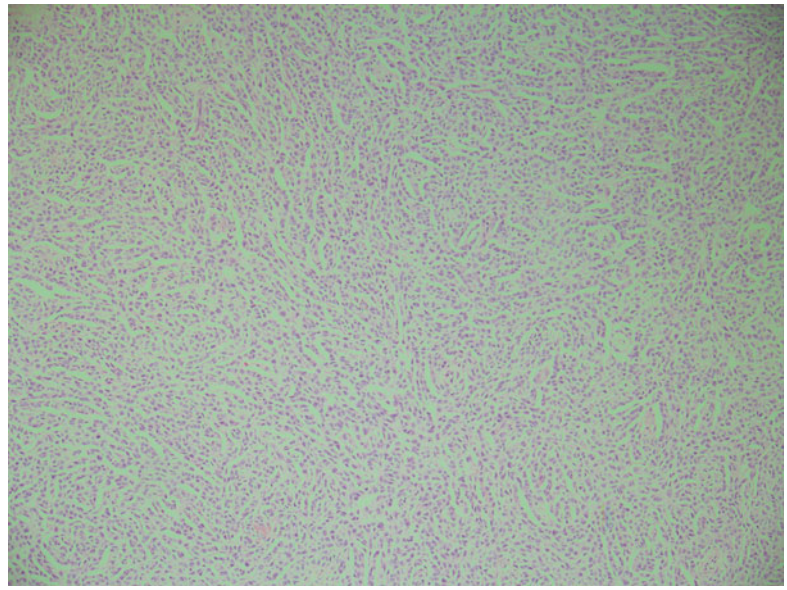

Fig. 15.3 Watered silk is the term given to this rippling pattern created by superimposed lines of cells in granulosa cell tumors (H&E ×100 magnification)

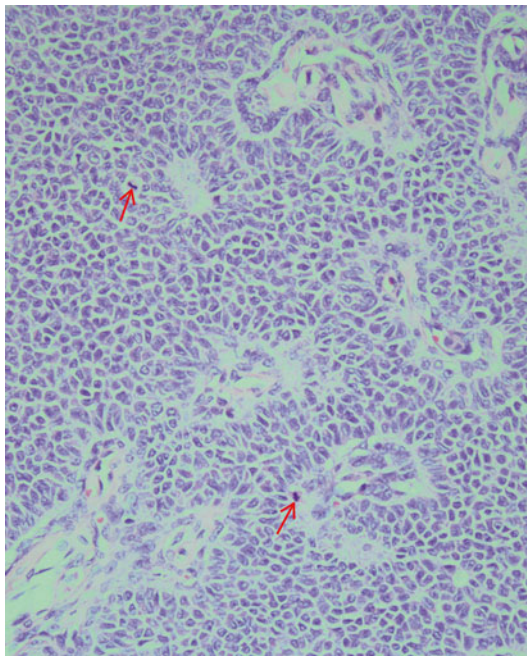

Fig. 15.4 Granulosa cell tumors can show brisk mitotic (*arrows*) activity (H&E ×200 magnification)

[21]. Cystic change in GCT (Fig. 15.7) is also likely to be a degenerative phenomenon. The diffuse pattern of AGCT is characterized by intersecting fascicles of cells with slightly spindle-shaped nuclei and scant cytoplasm. Such cases can be difficult to distinguish from fibromas and should prompt careful search for foci of typical AGCT cells with characteristic pale vesicular nuclei, with or without grooves [22]. AGCTs may contain heterologous elements, hepatic cells, and Leydig cells [23]. True sarcomatous change has been rarely described in AGCTs. In these tumors, focal areas of markedly pleomorphic cells with atypical mitoses, sometimes resembling carcinoma, are present in a background of otherwise typical AGCT [24]. This should be distinguished from a diffuse AGCT which is sometimes erroneously described as sarcomatoid [22].

AGCTs are typically positive for inhibin, calretinin, and CD56. Epithelial membrane antigen (EMA) is negative. This profile is retained in recurrent tumors [25, 26]. Low molecular weight cytokeratin can be positive in AGCTs. Recently, FOXL2 has been found to be a specific marker for AGCTs. JGCTs are not FOXL2 positive [27]. FOXL2 gene encodes a member of the forkhead domain/winged-helix family of transcription factors and is the earliest identified sexually dimorphic marker of ovarian differentiation. The finding that the somatic *FOXL2* C134W mutation is characteristic of AGCT is an advance in clinical molecular oncology [28, 29]. At a molecular level, the consequence of the mutation and its

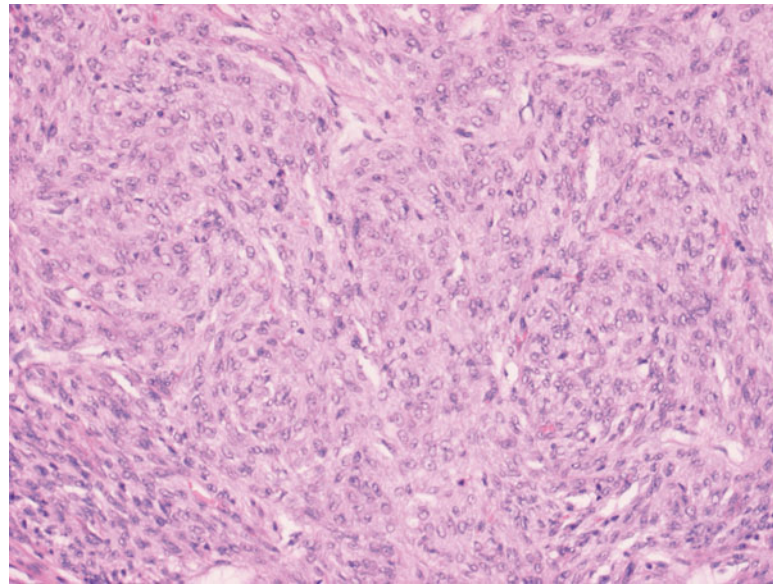

Fig. 15.5 The cytoplasm of the tumor cells in luteinized granulosa cell tumors is abundant. Some of the nuclei contain grooves (H&E ×200 magnification)

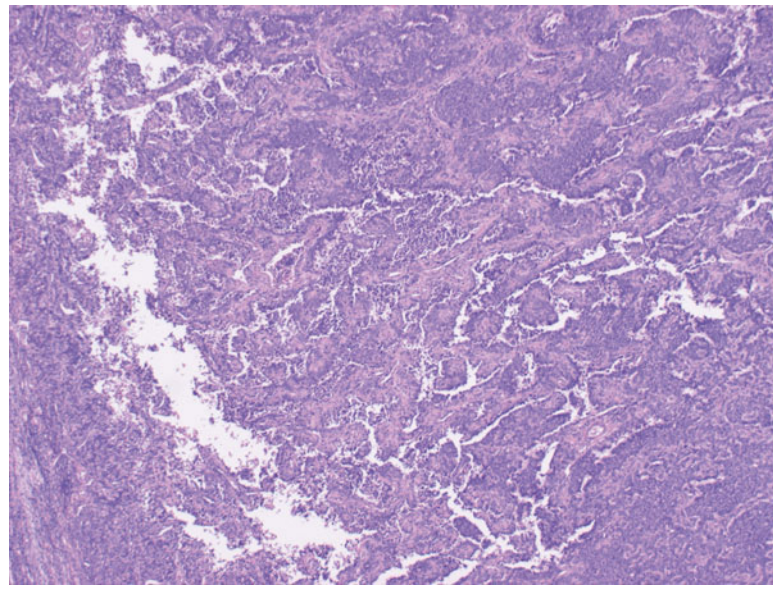

Fig. 15.6 This pseudopapillary pattern is a degenerative phenomenon within an adult granulosa cell tumor resulting in solid areas falling apart and resembling papillae (H&E ×100 magnification)

contribution to the mechanisms of GCT pathogenesis remain to be determined.

Differential Diagnosis

Thecoma–fibroma – this may be confused with a diffuse pattern of AGCT. Typically, tumors of the thecoma–fibroma group do not show the multipatterned morphology of AGCT, do not stain strongly for inhibin, and show a pericellular reticulin distribution. AGCT shows reticulin fibrils surrounding nests of cells (Fig. 15.8).

Transitional or undifferentiated carcinoma especially when there is a prominent pseudopapillary morphology in the AGCT. Microcystic spaces and slit-like fenestrations within thick bands of neoplastic epithelium are commonly seen in transitional cell carcinomas, but not in GCT [21]. Immunohistochemically, AGCTs are typically

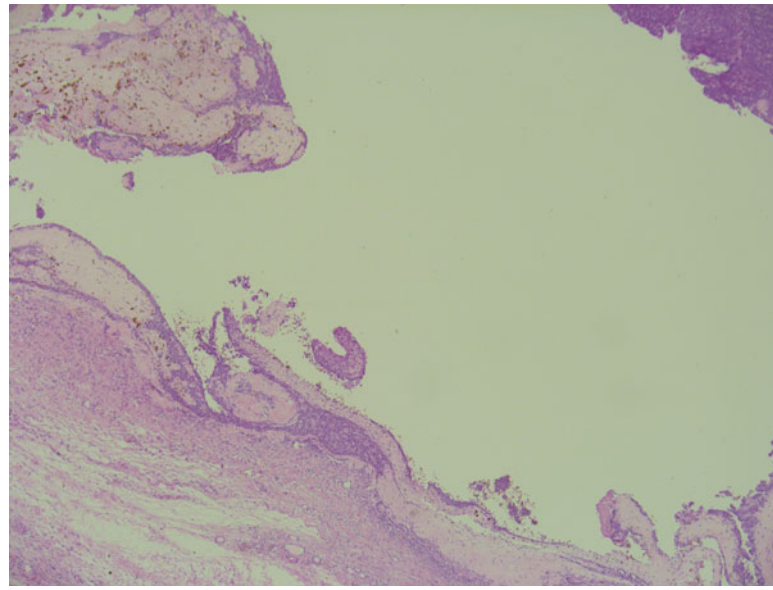

Fig. 15.7 Cystic granulosa cell tumor (H&E ×40 magnification) (H&E ×400 magnification)

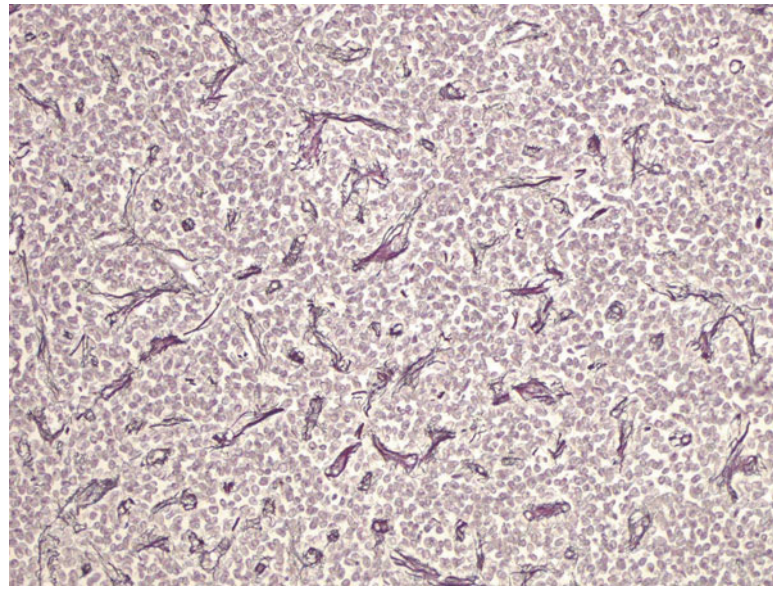

Fig. 15.8 In granulosa cell tumors, reticulin fibrils surround nests of cells. Occasionally, intercellular spaces have fragmented fibrils sometimes referred to as "broken twigs" (H&E ×200 magnification)

calretinin and inhibin positive with lack of EMA expression and the converse is true for carcinomas

Endometrial stromal sarcoma (ESS) with sex cord differentiation may pose problems as the cells of ESS may mimic AGCT with their monomorphism, scanty cytoplasm, and mitotic activity. However, they have numerous arterioles and are inhibin and calretinin negative. CD10 is not useful as AGCTs may also be positive with this marker.

Ovarian small cell carcinoma of hypercalcemic type can have a diffuse and micro-/macrofollicular pattern and on low power can be easily be mistaken for AGCT. However, monomorphic cells, grooved nuclei, relatively lower mitotic activity, and positive staining for inhibin help to define AGCT. Rare metastatic tumors such as small cell melanoma and lobular carcinoma of the breast need to be considered in the differential. Melanomas, unlike AGCTs, are HMB45 and S100 positive. Lobular carcinomas are EMA positive and may show intracytoplasmic mucin.

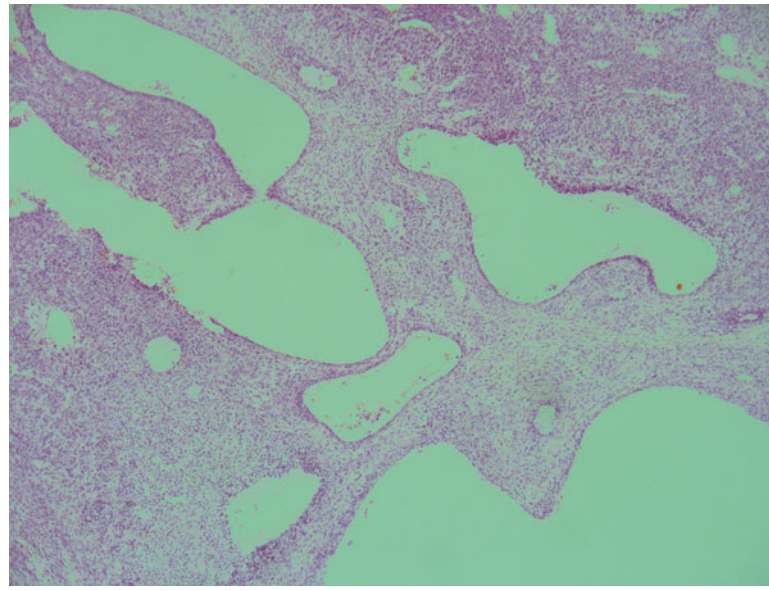

Fig. 15.9 Follicle-like spaces of varying sizes are typically seen in juvenile granulosa cell tumors (H&E ×100 magnification)

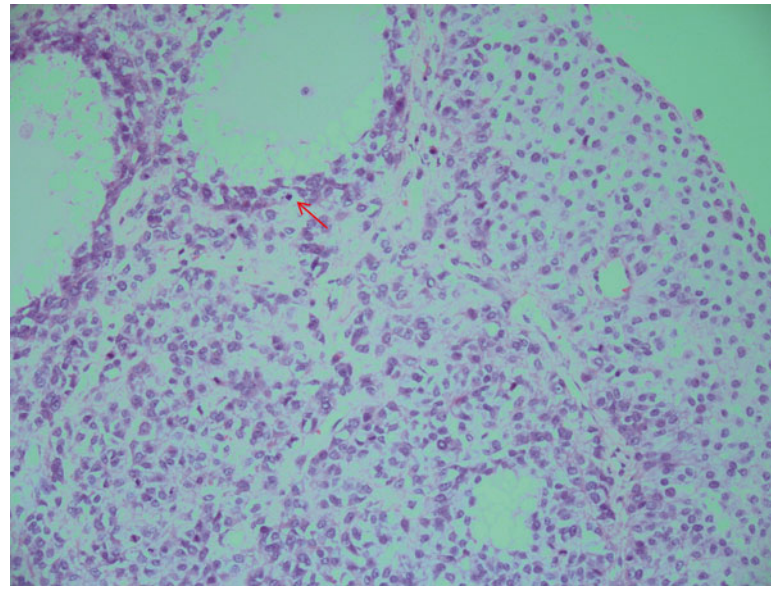

Fig. 15.10 Unlike the adult type, the nuclei in juvenile granulosa cell tumors do not have nuclear grooves. They often have conspicuous nucleoli. Mitotic figures (*arrowed*) are easily seen (H&E ×200 magnification)

Incidental granulosa cell proliferations can be seen in ovaries from pregnant women. These are microscopic lesions that are multiple and confined to regions in and around atretic follicles, features that help differentiate from AGCTs.

JGCTs

A characteristic feature of JGCTs is the presence of follicle-like spaces of varying shapes and sizes that contain secretions which may be eosinophilic or amphophilic (Fig. 15.9). The lining cells have rounded, non-grooved, hyperchromatic nuclei with abundant eosinophilic or vacuolated cytoplasm (Fig. 15.10). Random cytological atypia is not uncommon [30]. Mitotic activity is often brisk. The neoplasms may show a diffuse pattern and this can be associated with extensive luteinization of granulosa and theca cells [29]. The stroma can be myxoid or edematous. Uncommon findings include pseudopapillae [21], admixed foci of AGCT, and hyaline globules.

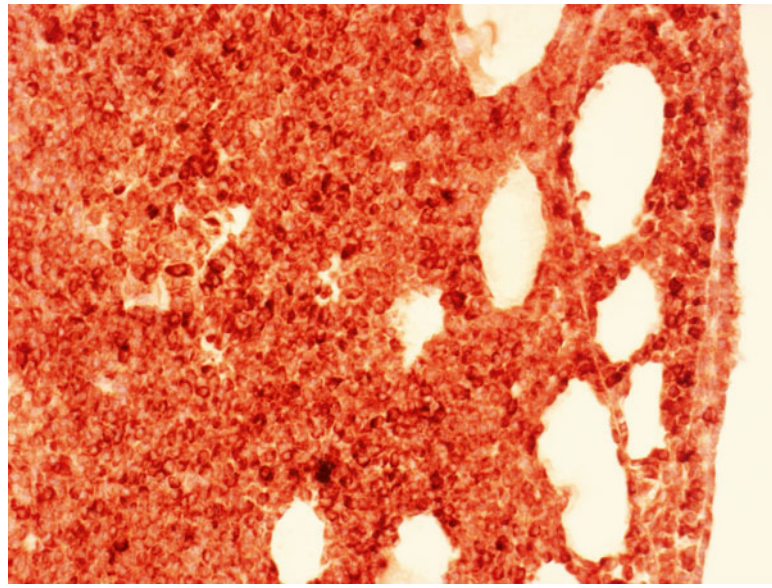

Fig. 15.11 The cells are positive for calretinin (H&E ×200 magnification)

Tumor cells are positive for inhibin, calretinin, vimentin, and CD56 (Fig. 15.11). They may also show positive staining for SMA, S100, CD99, and CD10. Unlike AGCTs, JGCTs can show EMA positivity in many cases [31]. FOXL2 staining is negative.

Differential Diagnosis

The salient differences with AGCT are the lack of nuclear grooving, presence of abundant cytoplasm, lesser heterogeneity of architecture, stromal luteinization, typically myxoid or edematous background, and intrafollicular secretions that are watery and amphophilic. Immunohistochemistry is similar except for EMA positivity in many JGCTs.

Large cell variant of small cell carcinoma of hypercalcemic type is an important differential, and rare JGCTs have been associated with raised serum calcium levels [32]. However, small cell carcinomas do not have hormonal manifestations, have greater mitotic activity, and are negative for inhibin.

Yolk sac tumors (YSTs) can be considered in the differential as they occur in young patients and can have an acinar pattern of growth. Cells constituting JGCT can show clearing of cytoplasm and may appear hob nailed mimicking YSTs. However, YSTs have more primitive appearing cells and stroma and are positive for AFP and glypican-3 [33].

An algorithmic approach to the diagnosis of ovarian tumors with follicular and cystic pattern is provided in Fig. 15.12.

Tumors of the Thecoma-Fibroma Group

Fibroma

Epidemiology and Clinical Correlates

Fibromas make up approximately 4 % of ovarian neoplasms. They occur in middle-aged women but can be seen in any age group. They are commonly unilateral and present with symptoms of a pelvic mass. Raised CA125 levels are often noted [34]. They are mostly hormonally inert. Bilateral or childhood ovarian fibromas should raise the possibility of Gorlin syndrome [35]. They are mostly hormonally inert. Ascites is seen in more than 10 % of cases, especially in tumors exceeding 10 cm in size. Meigs syndrome, defined as ascites and hydrothorax, occurs in approximately 1 % of patients [36].

Macroscopy

Fibromas have a smooth, lobulated surface and a firm, white uniform cut surface. Foci of edema

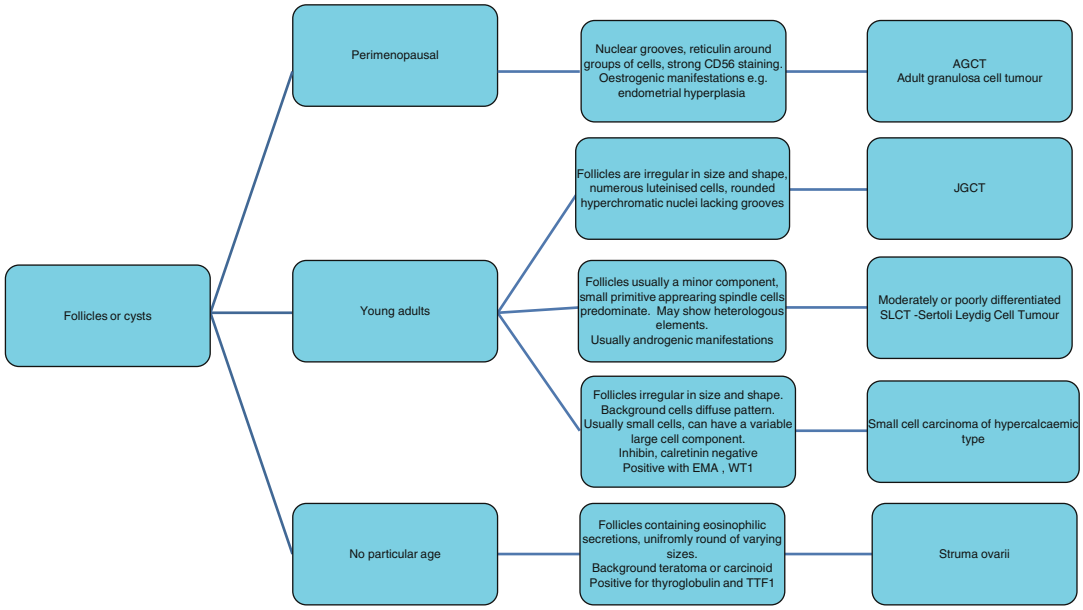

Fig. 15.12 An approach to ovarian sex cord-stromal tumors with follicular and cystic pattern

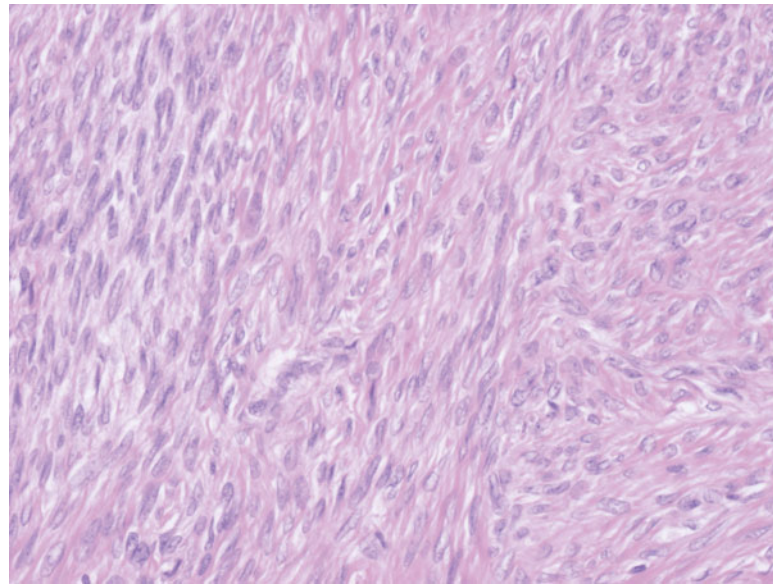

Fig. 15.13 Typical fibromas are composed of bland spindle cells arranged in intersecting fascicles. Note the collagen intervening between the cells (H&E ×200 magnification)

and calcification are seen. Extensive calcification is seen when there is associated Gorlin syndrome [37]. Cellular fibromas generally have a softer, tan to yellow cut surface.

Microscopy

They are composed of spindle cells with ovoid/elongated nuclei arranged in intersecting fascicles with occasional storiform areas (Fig. 15.13). The cells produce collagen. Edema, hyaline plaques, scattered luteinized cells, and small calcified foci can be seen. Mitoses are not conspicuous. Some fibromas can show the presence of sex cord-like elements in the form of tubules or cords (Fig. 15.14). When these constitute less than 10 % of the tumor, they are referred to as ovarian

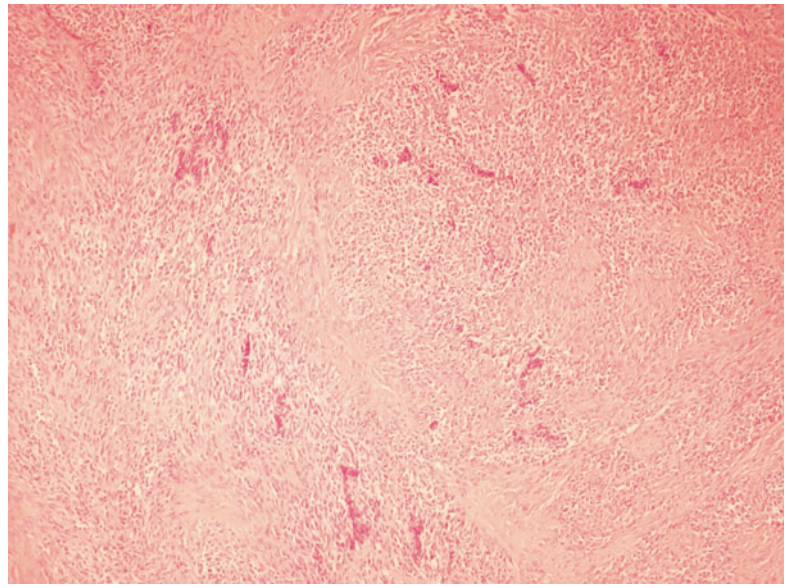

Fig. 15.14 In a small number of cases, fibromas can contain short cords or tubules representing minor sex cord elements (H&E ×40 magnification). Inset shows minor sex cord elements at higher magnification (H&E ×200 magnification)

fibromas with minor sex cord elements and have no prognostic significance [38]. Typical fibromas do not pose diagnostic difficulties and immunohistochemistry is not needed for diagnosis. About a third shows positive immunoreactivity for/with inhibin, calretinin, and CD56 [39].

About 10 % of fibromas are densely cellular and are designated cellular fibromas (CF). Of these, some have mitotic activity greater than 4/10 HPFs and have been designated mitotically active cellular fibromas (MACF) [40]. They are more often associated with extraovarian disease at the time of presentation and with ovarian surface adhesions. The importance of recognizing these variants is to distinguish them from the rare fibrosarcoma. Although CFs and MACFs are typically associated with a favorable outcome, there has been one reported case of death [41]. Some authors consider that CFs and MACFs especially those with extraovarian disease or adhesions should be sampled thoroughly to exclude a sarcomatous component and require long-term follow-up [22, 42].

Ovarian fibrosarcomas are large tumors showing extensive necrosis and hemorrhage and are characterized by moderately to severely atypical spindle cells with high mitotic counts. They are associated with a fatal outcome within 2 years in more than 50 % of reported cases [43, 44].

Differential Diagnosis

Edematous fibromas can be confused with sclerosing stromal tumors. A lobular pattern, dual cell population, and hemangiopericytomatous vessels are features of sclerosing stromal tumor that are not seen in fibromas. The latter usually have hyaline plaques.

Endometrial stromal sarcomas (ESSs) of the ovary can have fibromatous areas and cause problems in diagnosis. Thorough sampling will reveal the typical arteriolar pattern in ESSs. They are typically negative for inhibin and calretinin.

Fibromas with minor sex cord elements can be rarely confused with ESSs with sex cord elements or synovial sarcomas. The latter show high-grade nuclear atypia and brisk mitotic activity. The tubular and cord-like structures in fibromas with minor sex cord elements are positive for inhibin and calretinin, while similar structures in synovial sarcomas are positive for EMA and other epithelial markers.

Thecoma

The average age of patients with typical thecomas is 53 years. Most have estrogenic manifestations that lead to the discovery of ovarian tumor. In the largest series [45], a fifth of patients had concomitant endometrial cancers.

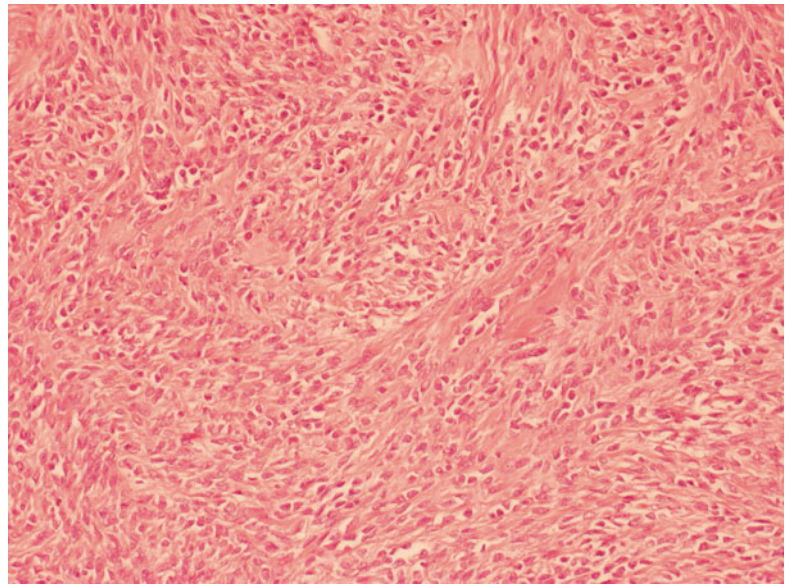

Fig. 15.15 Luteinized thecomas show a fibroma-like background with variable-sized clusters of luteinized cells with clear cytoplasm (H&E ×200 magnification)

Overall, typical thecomas are benign. They are solid tumors with a smooth capsule and typically yellow cut surface reflective of their lipid content. On microscopy, they are composed of uniform cells with bland nuclei and abundant pale to vacuolated cytoplasm. Hyaline plaques may be noted.

Luteinized Thecomas (LTs)

LTs occur in women who on average are slightly younger than patients with typical thecomas. About 50 % of the women display estrogenic manifestations and nearly a fifth may be virilized due to their androgenic effect. LTs may recur and can rarely be fatal [46]. Some LTs are associated with sclerosing peritonitis [47, 48]. In patients with sclerosing peritonitis, the latter may be associated with significant morbidity. On macroscopy, they appear similar to typical thecomas. On microscopy, there is a fibroma-like background with variably sized clusters of luteinized cells (Fig. 15.15). There can be an overlap in the appearances of fibromas and thecomas. Many use the term fibrothecoma to encompass these neoplasms. They however have distinct clinical and morphological profiles. Retaining the term thecoma for the neoplasms that have cells with abundant vacuolated cytoplasm and using the term fibroma for all others would aid delineation of these clinically distinct entities. Luteinized thecoma is perhaps a misnomer as it typically indicates a fibromatous neoplasm with a component of luteinized cells [42]. LTs associated with sclerosing peritonitis are more cellular, mitotically active, and contain smaller luteinized cells. They have a microcystic appearance due to edema and entrap rather than replace preexistent ovarian follicles. These LTs are different and some authorities consider the ovarian lesion in these cases to be nonneoplastic. The background cells are often negative for sex cord–stromal markers while these are expressed by the luteinized cells [49].

Cellular Thecomas and Mitotically Active Cellular Thecomas

Cellular and mitotically active thecomas including luteinized thecomas have been described applying the criteria used for similar variants of ovarian fibromas.

However, due to the low numbers, it is not possible to define their behavior. In general, careful clinical evaluation and follow-up is advised in cases with rupture, extraovarian disease, or adhesions at diagnosis. Another rare variant of thecomas is the calcified thecoma [50], which occurs in young women and has been associated with a benign outcome.

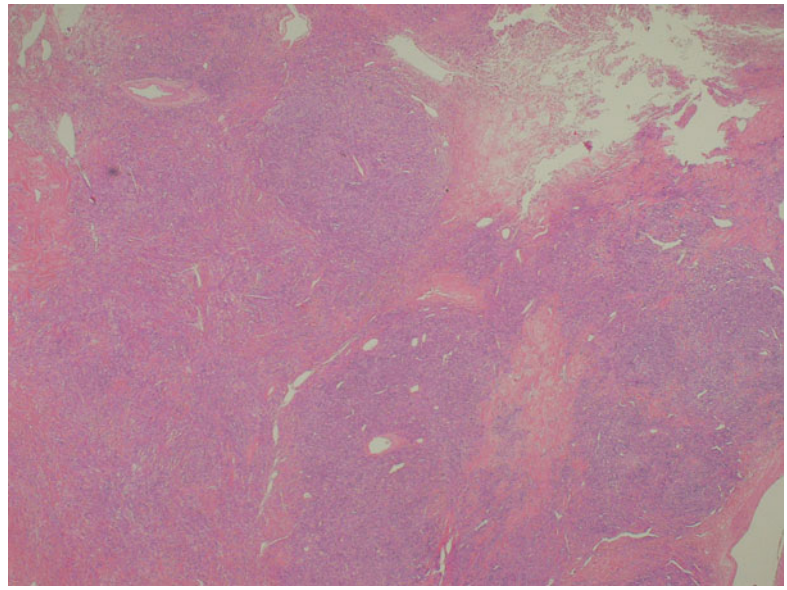

Fig. 15.16 Sclerosing stromal tumors are characterized by lobules of cellular areas which are separated by edematous or collagenized stroma (H&E ×40 magnification)

Malignant Thecomas

These are rarely described encountered. The diagnosis must be postulated in cases with significant nuclear atypia and high mitotic index [41, 47].

Sclerosing Stromal Tumor

Sclerosing stromal tumor is an uncommon benign tumor that mostly occurs in women under 30 years of age [51]. They are mostly unilateral and hormonally inactive but can be associated with symptoms of androgen or estrogen excess. The former have also been reported in pregnancy [52, 53]. All patients have had an uneventful follow-up. Typically, the tumor is solid, gray white with occasional yellow areas on cut surface. Rarely, unilocular cysts can be seen. The tumor has a characteristic low-power appearance. Lobules of cellular areas are separated by hypocellular areas containing edematous or collagenized stroma (Fig. 15.16). The cellular lobules have thin-walled, dilated, hemangiopericytoma-like vessels that have "staghorn" outlines (Fig. 15.17). Within the cellular areas, there is a mixture of small spindle cells and luteinized cells that have uniform nuclei with conspicuous nucleoli and eosinophilic or vacuolated cytoplasm (Fig. 15.18). When vacuolated, these cells can resemble signet ring cells. Tumors are typically cytokeratin negative, vimentin positive, and positive for markers of sex cord–stromal differentiation, including inhibin, calretinin, and Melan-A [42] (Fig. 15.19).

Differential Diagnosis. The tumor can be confused with signet-ring stromal tumor. Signet-ring stromal tumors have a homogenous appearance. Immunohistochemistry is not helpful.

The other differential is a metastatic carcinoma with signet ring morphology. In these, the signet ring cells contain intracytoplasmic mucin and can be identified as being epithelial cells by immunohistochemical stains.

Signet-Ring Stromal Tumor

This is a rare neoplasm occurring in adult women and is most commonly unilateral. There are no reported endocrine manifestations or associations with aggressive behavior [54]. Macroscopically, the sectioned surface is solid or solid and cystic. The tumor is composed of cells with bland eccentric nuclei and a single large cytoplasmic vacuole set is a fibromatous background (Fig. 15.20). The vacuoles do not contain mucin, glycogen, or lipid. They have been shown to contain cytoplasmic pseudoinclusions of edematous extracellular matrix [55]. The cells are positive for vimentin and smooth muscle actin. They do not mark with epithelial membrane antigen.

The important differential diagnosis is a Krukenberg tumor [56, 57]. Signet-ring stromal

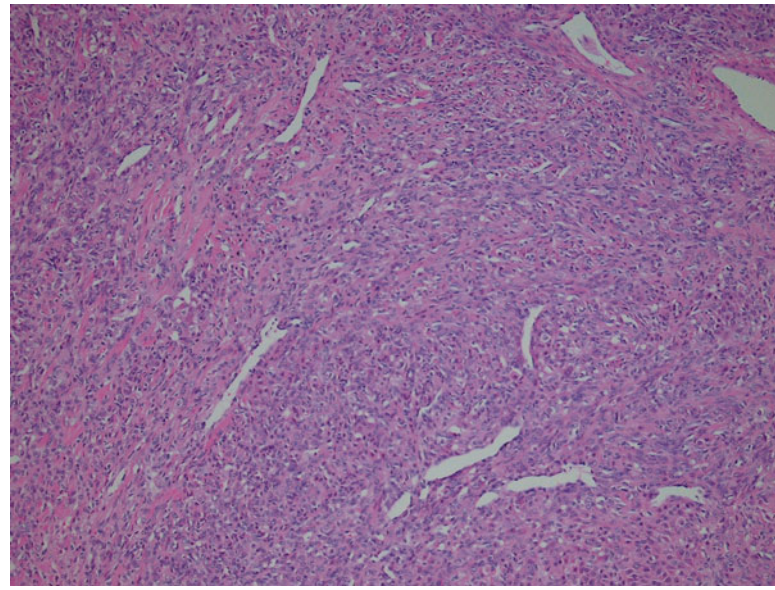

Fig. 15.17 Staghorn-shaped hemangiopericytomatous vessels are typically present (H&E ×200 magnification)

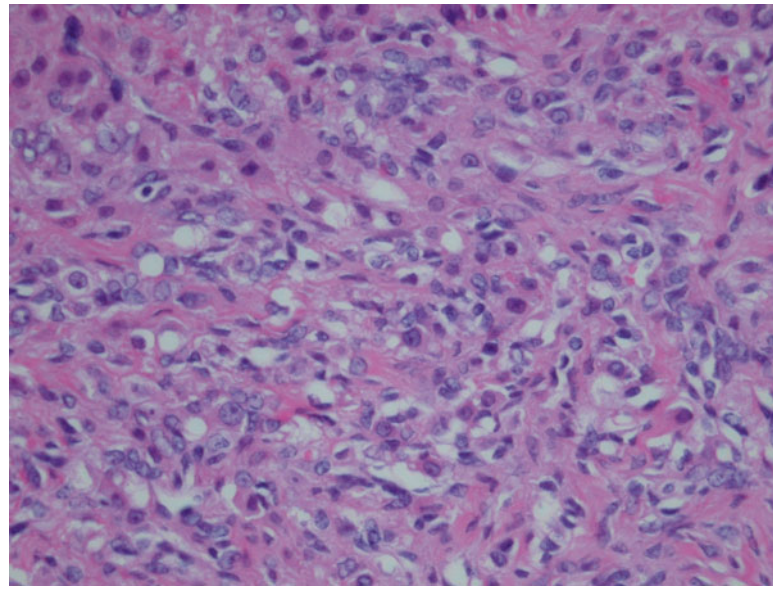

Fig. 15.18 Sclerosing stromal tumors are composed of a mixture of small spindle cells and luteinized cells with eosinophilic or vacuolated cytoplasm (H&E ×400 magnification)

tumors are commonly unilateral and do not show any gland formation or trabecular architecture. The cells are bland and lack mucin or immunohistochemical expression of epithelial differentiation.

Microcystic Stromal Tumor

This is a recently described, presently rare, unilateral ovarian tumor of women of reproductive age that lacks hormonal manifestations and presents as a pelvic mass [58]. They are large solid, cystic, or solid–cystic tumors. The tumor is lobulated and sharply demarcated from the surrounding ovarian stroma. It consists of three components in varying proportions: microcysts, solid cellular zones, and fibrous stroma. The nuclei are small and uniform with indistinct nucleoli. Mitotic activity is low. The characteristic immunophenotype is positive staining for CD10 and vimentin and negative staining for

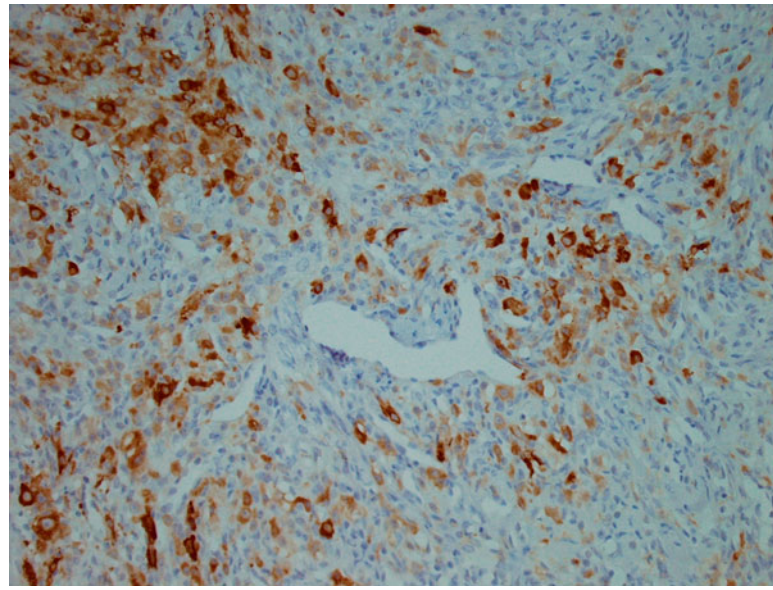

Fig. 15.19 The neoplastic cells in sclerosing stromal tumors are positive for calretinin (H&E ×200 magnification)

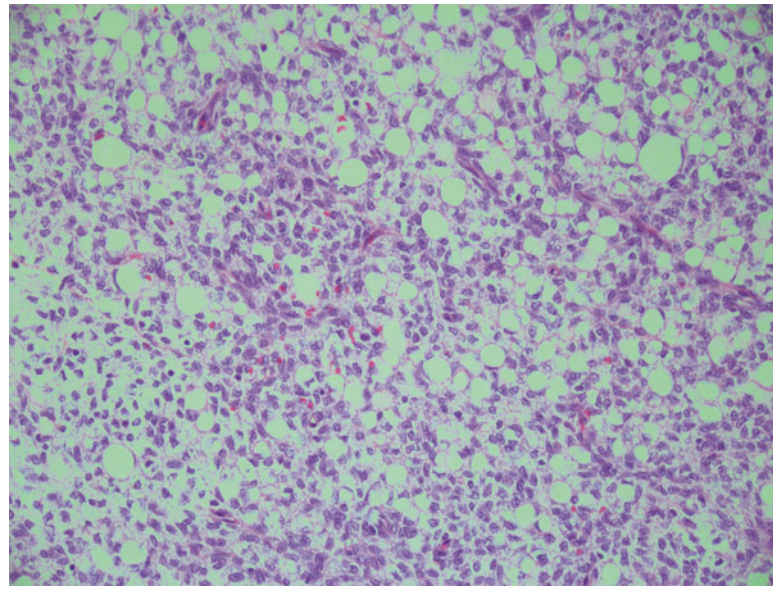

Fig. 15.20 Signet-ring stromal tumors contain bland cells with a single cytoplasmic vacuole (H&E ×400 magnification)

epithelial membrane antigen. Inhibin and calretinin are usually negative [59]. A recent study has shown an oncogenic mutation that strongly suggests dysregulation of the Wnt/β-catenin pathway [60].

Differential Diagnosis. Thecomas and LTs with sclerosing peritonitis can show a prominent microcystic growth pattern. They are however typically bilateral and have hormonal manifestations, and lesional cells are diffusely positive for CD56, inhibin, and calretinin.

Germ cell tumors including yolk sac tumor (YST) and teratomas have a variable microcystic pattern. Ample sampling is necessary to rule out more specific patterns of other neoplasms. Immunohistochemistry for YST is diagnostically distinct.

Ovarian myxomas are rare neoplasms of presumed ovarian stromal origin that consist of bland stellate cells in a loose myxoid matrix that has a delicate vasculature. The cells have an SMA-positive, desmin-negative myofibroblastic

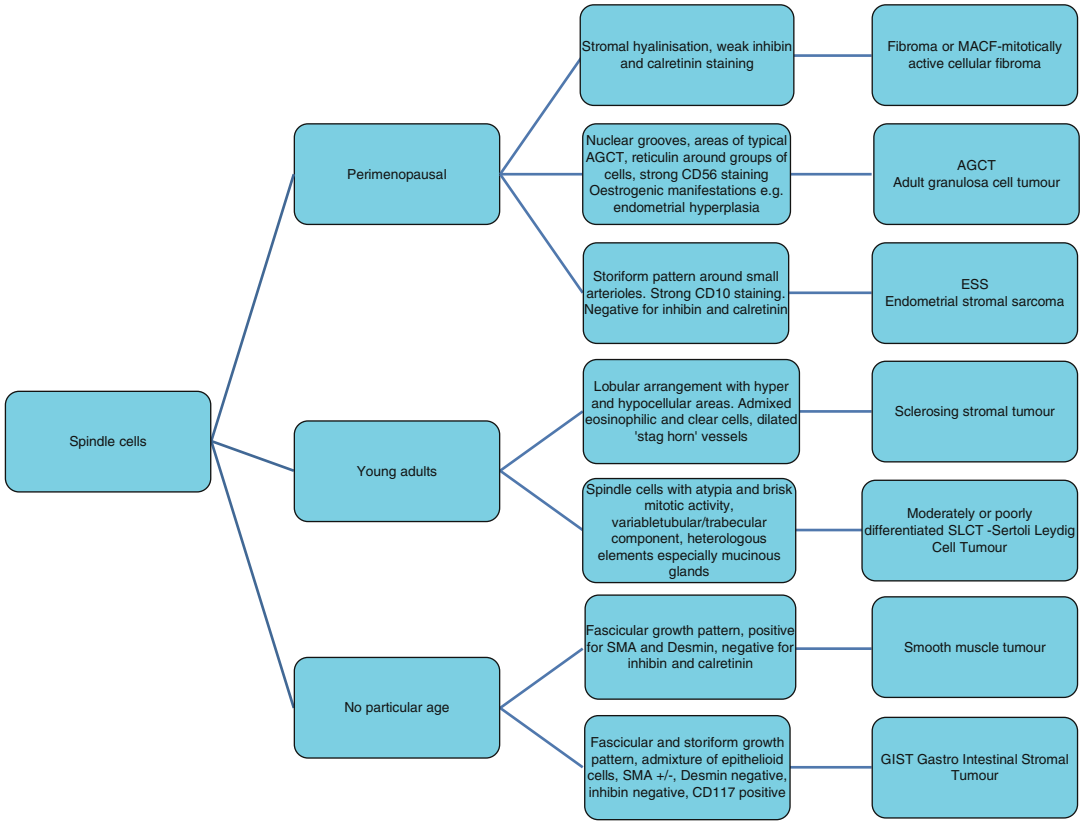

Fig. 15.21 An approach to spindled ovarian tumors with focus on sex cord–stromal tumors

immunoprofile and the myxoid matrix is Alcian blue positive [61].

An algorithmic approach to ovarian tumors with a dominant spindled pattern is seen in Fig. 15.21.

Sertoli–Stromal Tumors

Sertoli–stromal cell tumors are those that contain Sertoli cells, Leydig cells, and fibrocytes in varying proportions and varying differentiation. They are generally rare tumors, the most common being Sertoli–Leydig cell tumors.

Sertoli–Leydig Cell Tumors

Epidemiology and Clinical Correlates

They constitute less than 0.5 % of all ovarian tumors. The average age of occurrence is around 25 years, with the well-differentiated type occurring a decade later and the retiform variant occurring a decade earlier [62]. About a third of patients present with virilizing symptoms. Less commonly hyperestrogenic symptoms, raised serum alpha-fetoprotein (AFP) [63] or CA125 levels, are noted. Well-differentiated SLCTs tend to behave in a benign fashion. Features that indicate poor prognosis are retiform histology, poor differentiation, tumor rupture, and FIGO stage 1c or higher. In contrast to AGCTs, SLCTs recur early with very rare recurrences beyond 5 years [64].

Macroscopy

Sertoli–Leydig cell tumors (SLCTs) are generally unilateral. The tumors vary greatly in size and range from 5 to 20 cm. The poorly differentiated tumors and those with heterologous elements tend to be larger and contain areas of hemorrhage and necrosis [62]. Retiform variants may have large cystic spaces with papillary projections simulating borderline serous tumors

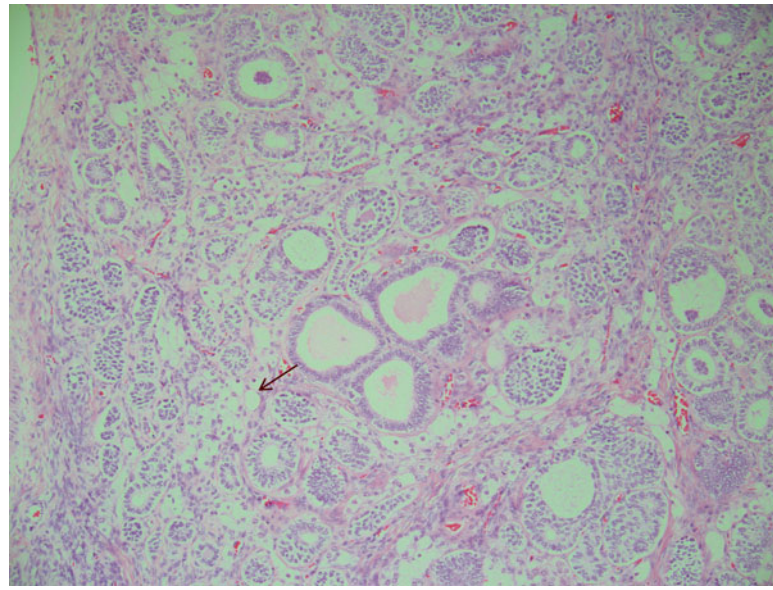

Fig. 15.22 This is a well-differentiated Sertoli–Leydig cell tumor composed of Sertoli tubules of varying sizes with intervening vacuolated Leydig cells (*arrowed*) (H&E ×100 magnification)

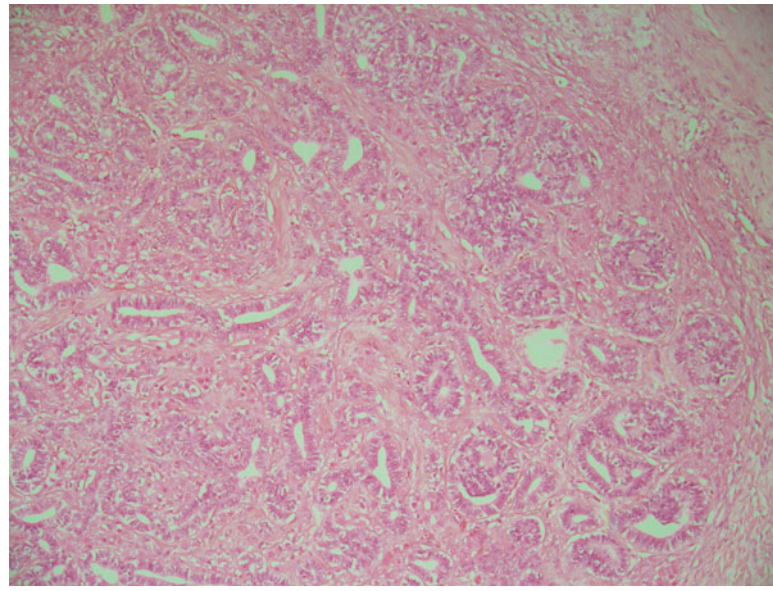

Fig. 15.23 The tubules lined by Sertoli cells can mimic an endometrioid pattern (H&E ×100 magnification)

[65]. Most SLCTs have soft, lobulated, yellow, or tan cut surfaces.

Microscopy

SLCTs are classified into five major categories based on clinical and pathological differences: (1) well-differentiated, (2) intermediate differentiated, (3) poorly differentiated, (4) retiform, and (5) tumors with heterologous elements.

Well-differentiated SLCTs are composed of tubules of varying sizes separated by a fibromatous stroma containing Leydig cells (Fig. 15.22). Rarely, the tubules may have an endometrioid appearance [66] (Fig. 15.23). The Sertoli cells lining the tubules are columnar with basal nuclei and abundant eosinophilic or vacuolated cytoplasm. The Leydig cells are single or clustered and have variably vacuolated cytoplasm. Cytochrome pigment can be present. Reinke

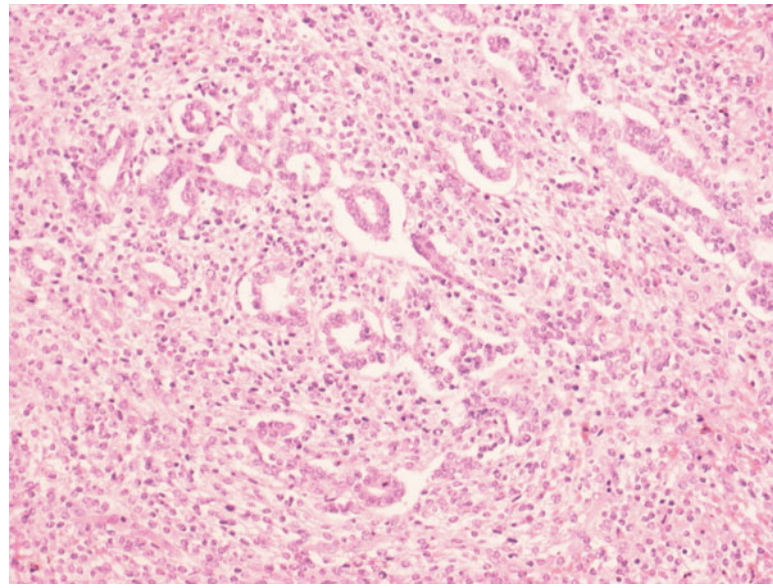

Fig. 15.24 Poorly formed Sertoli tubules are seen in an edematous stroma containing Leydig cells. This is a Sertoli–Leydig cell tumor of intermediate differentiation (H&E ×200 magnification)

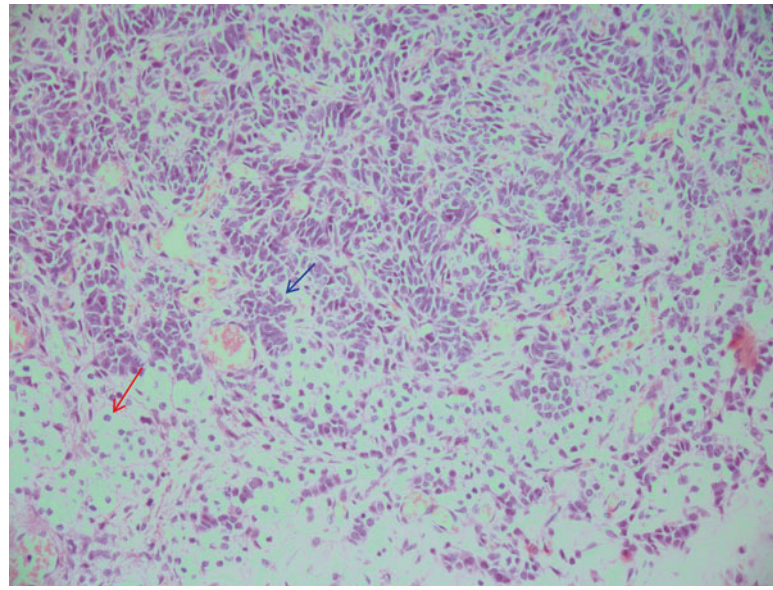

Fig. 15.25 Poorly formed cords and diffuse sheets of Sertoli cells (*blue arrow*) and vacuolated Leydig cells (*red arrow*) constitute this Sertoli–Leydig cell tumor of intermediate differentiation (H&E ×200 magnification)

crystals may be identified. Cytological atypia and mitotic activity are rare.

Intermediately and poorly differentiated SLCTs have a variety of patterns that form a morphological continuum. The intermediately differentiated tumors often have a striking lobular architecture. The cellular areas are composed of immature Sertoli cells. They are small and spindled, have scanty cytoplasm, and show mitotic activity. They are arranged in cords, nests, columns, diffuse sheets, or poorly formed tubules (Figs. 15.24 and 15.25). Infrequently, cyst-like spaces with eosinophilic material can also be seen. The stroma separating the Sertoli cell component can be fibromatous, edematous, or densely cellular. The stroma may focally resemble immature mesenchyme and have a sarcomatous appearance, especially dominant in poorly differentiated tumors (Fig. 15.26). Leydig cells are typically present.

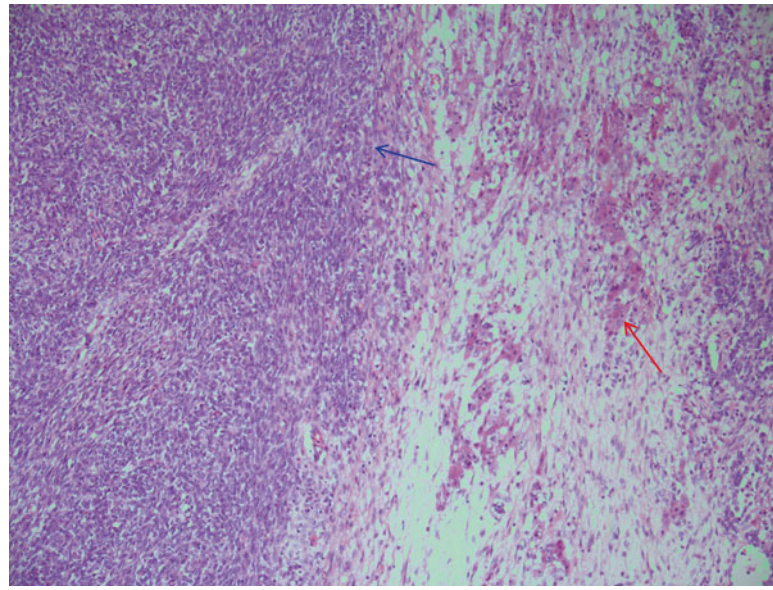

Fig. 15.26 This tumor has spindled Sertoli cells that resemble immature mesenchyme (*blue arrow*) with a sarcomatous appearance typical of poorly differentiated tumors. Its Sertoli–Leydig cell nature is revealed by the presence of Leydig cells (*red arrow*) (H&E ×200 magnification)

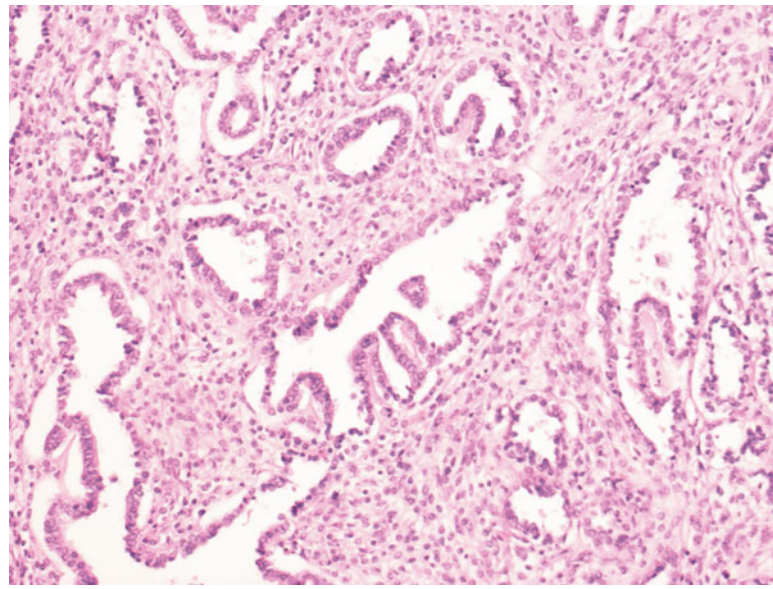

Fig. 15.27 Retiform SLCTs show irregularly branching tubules with intraluminal papillae with Leydig cells in the intervening stroma (H&E ×200 magnification)

Retiform SLCTs [67–69] show, on low power, irregularly branching tubules and cysts with intraluminal papillae resembling the rete testis (Fig. 15.27). The papillae may have hyalinized or edematous cores and are covered by stratified cells. The stroma is similar to that seen in an SLCT of intermediate differentiation.

SLCTs may contain cysts and glands lined by mucinous epithelium containing goblet cells [70] (Fig. 15.28). Less commonly, type cartilage, primitive rhabdomyoblasts, neuroblastoma-like areas, and AFP-positive hepatocytes may be seen [71]. The latter account for cases of SLCTs associated with raised serum AFP levels. The hepatocytes can be overlooked as they can resemble Leydig cells.

Immunohistochemistry

SLCTs are positive for inhibin, calretinin, and CD56 [40] (Fig. 15.29). They typically lack staining for EMA. Retiform elements of SLCTs can be cytokeratin and EMA positive [69].

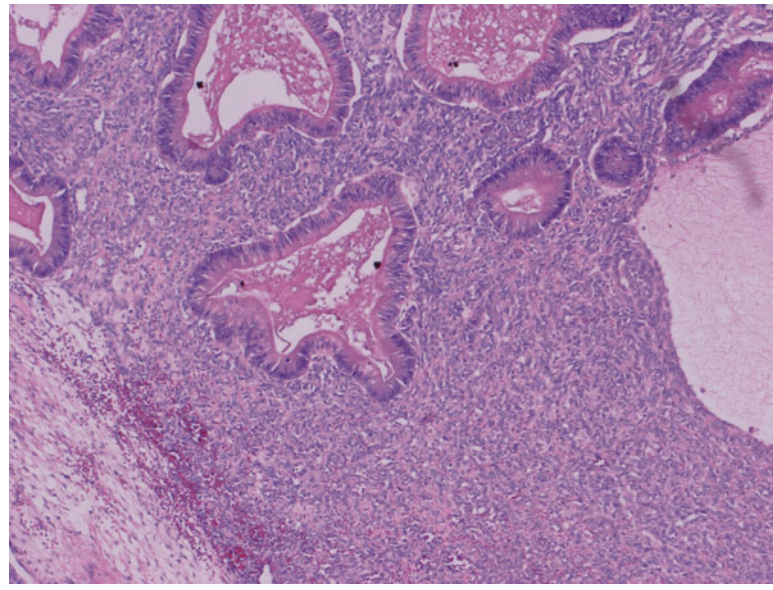

Fig. 15.28 Glands lined by mucinous epithelium are the most common manifestation of heterologous elements in Sertoli–Leydig cell tumors (H&E ×200 magnification)

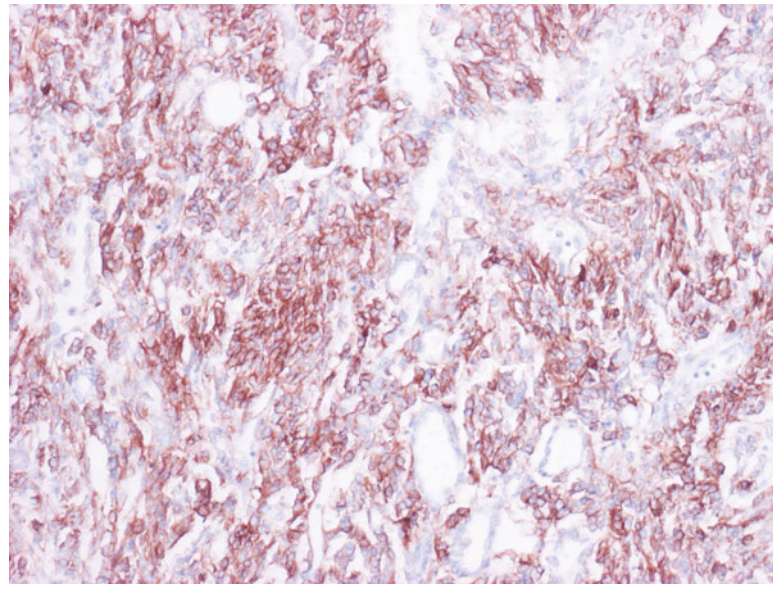

Fig. 15.29 Sertoli–Leydig cell tumors are CD56 positive (H&E ×200 magnification)

FOXL2 (402G->C) mutation has also been observed in SLCTs and can be detected immunohistochemically. SLCTs not staining for this marker tended to be retiform and poorly differentiated types [27].

Differential Diagnosis

Endometrioid carcinomas, when they have a sex cord-like pattern especially when they have luteinized stromal cells, can mimic SLCTs. In contrast to SLCTs, their sex cord-like structures are EMA positive and inhibin negative.

SLCTs can be mistaken for mucinous tumors if the sampling has mainly included the heterologous mucinous component. The intervening stroma is a clue as it is very cellular and primitive in appearance. Thorough sampling will reveal more typical areas.

Retiform tumors may be confused with serous epithelial tumors. The possibility of SLCT is greater with young age, presence of androgenic

effects, and typical areas of cellular stroma with Leydig cells. The cells lining the retiform areas are low grade and monomorphic in comparison to typical serous tumors.

Sarcomas and carcinosarcomas may show areas similar to poorly differentiated SLCTs. The latter can be a special problem when malignant cartilage is mistaken for heterologous elements. However, SLCTs generally occur in younger women, are not associated with malignant epithelial elements, and have typical immunoprofile.

Sertoli Cell Tumors

Epidemiology and Clinical Correlates

These are rare tumors occurring at any age (mean age 30 years). Hormonal manifestations, while not typical, can be estrogenic or androgenic. They are rarely associated with Peutz–Jeghers syndrome. They are typically unilateral and have a solid, yellow, or brown cut surface. The predominant pattern is tubular with rarer patterns including diffuse, retiform, and spindled. The cells lining the tubules may have eosinophilic or vacuolated cytoplasm. Mitotic activity is low. Tumors with pleomorphism, mitotic activity >5/10 HPF, and necrosis have malignant behavior and poor outcome [72]. Immunohistochemistry is similar to SLCTs.

Differential Diagnosis

Well-differentiated SLCT differs by the presence of Leydig cells in the stroma.

Tumors with diffuse patterns may mimic AGCT but lack the nuclear grooving and scanty cytoplasm typifying the latter.

Tumors with nested pattern and vacuolated cytoplasm can be mistaken for dysgerminomas but do not show stromal lymphocytes or CD117/PLAP/OCT3/4 immunoexpression of dysgerminomas.

Stromal–Leydig Cell Tumor

This is another rare tumor that typically occurs in postmenopausal women [46, 73]. Approximately half of the patients are virilized and a third show estrogenic manifestations. Younger, pregnant patients tend to show virilizing features [74]. Clinically, these are benign neoplasms. The cut surface of these tumors is often multinodular and multilobulated. The tumor is composed of nodules of Leydig cells within a neoplastic stromal proliferation arranged in a fibromatous background. By definition, intracytoplasmic refractile, cylindrical, rectangular/rhomboid structures – crystals of Reinke – must be identified to establish the diagnosis. The cells are positive for calretinin, inhibin, and CD56.

Differential Diagnosis

Luteinized thecoma is only distinguished by a lack of Reinke crystals.

Sex Cord–Stromal Tumors of Mixed or Unclassified Type

Sex Cord Tumors with Annular Tubules (SCTAT)

This unique neoplasm was originally described in 1970 by Professor Scully [75], and a series of 74 cases were reported 12 years later [76]. It occurs in two forms: as a multifocal, bilateral tumor in patients with Peutz–Jeghers syndrome or as a solitary neoplasm in individuals without evidence of the syndrome. About 30 % of patients with SCTAT have Peutz–Jeghers syndrome. Although syndromic SCTATs are mostly benign, about 15 % also harbor adenoma malignum of the cervix and some have malignancy in their gastrointestinal polyps. The tumor has a distinctive appearance with ring-shaped complex or simple tubules composed of cells with nuclei oriented peripherally and centrally around a hyaline body containing basement membrane material leaving an intervening anuclear cytoplasmic zone.

Genetics

Germline mutations in the tumor-suppressor *STK11* gene accompanied by loss of heterozygosity of markers near the wild-type *STK11* allele

were found in two Peutz–Jeghers syndrome-associated sex cord tumors with annular tubules, but they were not found in cases of sporadic sex cord tumor with annular tubules or minimal deviation adenocarcinoma studied [77].

Gynandroblastoma

This tumor is defined as one which contains both well-differentiated SLCT element and a granulosa cell tumor element, with the lesser component comprising at least 10 % of the neoplasm. Therefore, such a tumor may contain Call–Exner bodies as well as Sertoli cell tubules and Leydig cells in the stroma. Juvenile granulosa component has also been described [78]. Almost all reported cases are FIGO Stage 1 and have good prognosis.

Recently, [79] McCluggage and colleagues have shown that FOXL2 staining is not seen in the granulosa cell tumor appearing components of gynandroblastomas raising doubts on the origin of this component.

An algorithmic approach to ovarian sex cord–stromal tumors with tubular and pseudo-tubular pattern is presented in Fig. 15.30.

Steroid Cell Tumors

Steroid cell tumors include stromal luteomas, Leydig cell tumor, and steroid cell tumor not otherwise specified (NOS). These categories have overlapping clinical features. Stromal luteomas and Leydig cell tumors occur in postmenopausal women, while steroid cell tumors NOS occur over a wider age range. The latter two usually present with androgenic symptoms while hormonal manifestation of stromal luteomas is more often estrogenic. All three tumors are usually unilateral. Stromal luteomas and Leydig cell tumors are essentially benign while about half of steroid cell tumors NOS can be malignant [80].

Stromal Luteomas

These tumors are generally small and centered on the ovarian stroma (Fig. 15.31). The cells are polygonal and have eosinophilic cytoplasm often with lipochrome pigment. Crystals of Reinke are definitionally absent [81]. Degenerative changes may produce pseudoglandular spaces. Stromal hyperthecosis is seen in the same and/or opposite

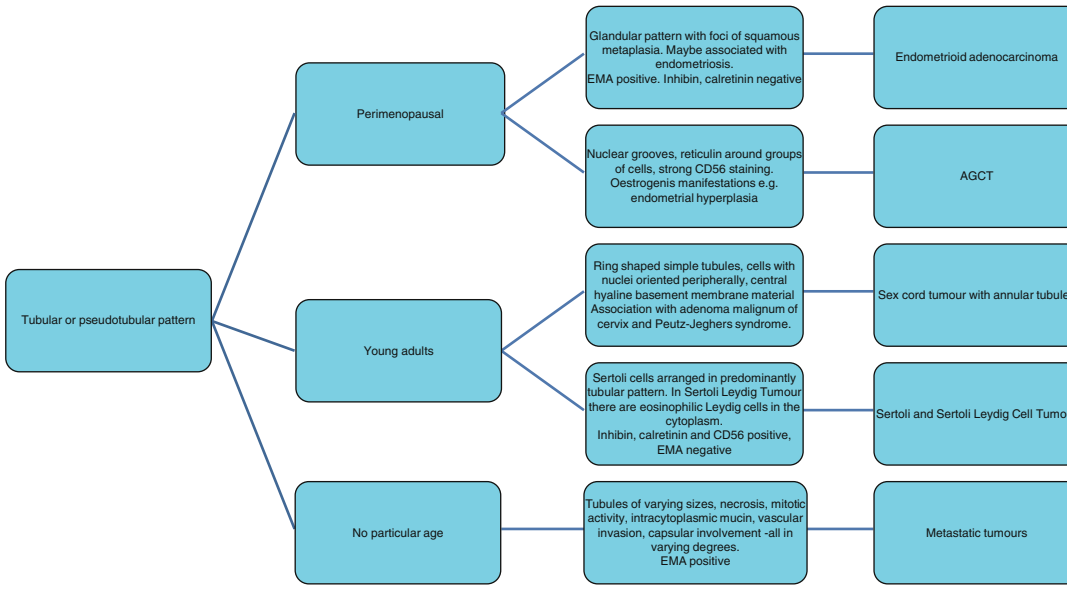

Fig. 15.30 An approach to ovarian tumors with tubular and pseudo-tubular pattern with focus on sex cord–stromal tumors

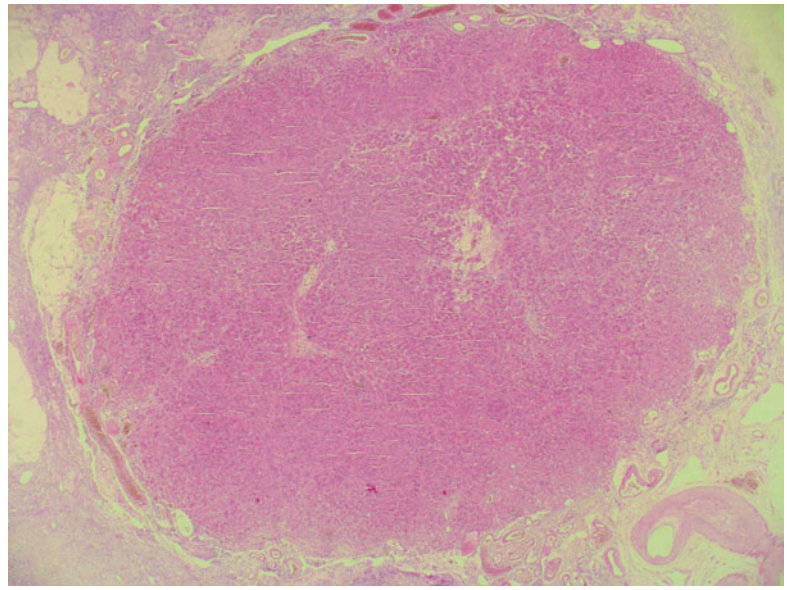

Fig. 15.31 This is a stromal luteoma, just over 5 mm in maximum dimension, well circumscribed, and surrounded by ovarian stroma (H&E ×40 magnification)

ovary. Nodular stromal hyperthecosis may be mistaken for stromal luteomas; a size of 5 mm is the arbitrary cutoff between the two lesions. The other differential is a non-hilar Leydig cell tumor which is typically androgenic and lacks Reinke crystals. Luteinized thecoma is differentiated by its occurrence in postmenopausal women and the finding of fibromatous stroma. Pregnancy luteomas can be identical to stromal luteomas. However, the history of pregnancy and multifocality of pregnancy luteomas helps in the differential.

Leydig Cell Tumors

Based on their location, they may be termed stromal or hilar Leydig cell tumors. The constituent cells are round or polygonal with small pyknotic nuclei and eosinophilic or clear cytoplasm. They have a diffuse or lobulated growth pattern and consist of cellular areas with clustering of small uniform nuclei separated by cytoplasmic zones. The stroma can be collagenous and vary in amount. Reinke crystals are definitionally present. However, a presumptive diagnosis of hilar Leydig cell tumor can be made even in the absence of identifiable crystals on the basis of a hilar location, a background of hilus cell hyperplasia, proximity to nonmedullated nerves, fibrinoid necrosis of blood vessel walls, or nuclear clustering with intervening nucleus-free zones [82].

Reinke crystals consist of pale eosinophilic rod-shaped crystals often seen within a retraction space [83]. The crystal is believed to be a protein product and is commonly seen in Leydig cells of the postpubertal testis.

Steroid Cell Tumors NOS

Tumors that do not have the diagnostic features of either stromal luteomas or Leydig cell tumors are categorized as steroid cell tumors NOS. They constitute the largest group in this category.

They generally have a diffuse growth pattern. The stroma is usually scant and associated with a network of thin-walled vessels. The cells are large with distinct cell membranes and granular eosinophilic or vacuolated cytoplasm (Fig. 15.32). Mitotic activity is generally low. Nearly half the cases were malignant in the largest series [80], and pathological features associated with adverse outcome were a size greater than 7 cm in diameter, areas of hemorrhage or necrosis, moderate to marked nuclear atypia, and a mitotic index of 2 or more per 10 high-power fields (Fig. 15.33). Nearly 20 % of patients present with advanced stage disease. Cytoreductive surgery may result

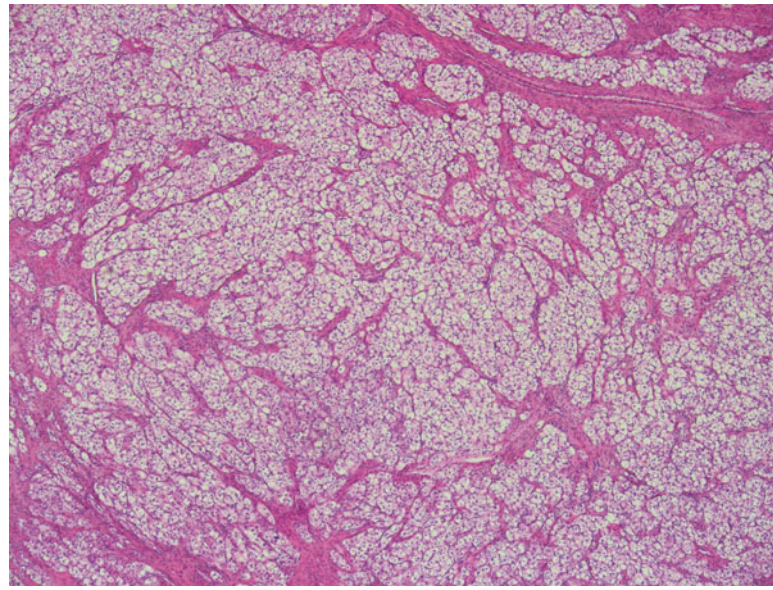

Fig. 15.32 Steroid cell tumors have a diffuse growth pattern. The cells usually have finely vacuolated cytoplasm (H&E ×40 magnification)

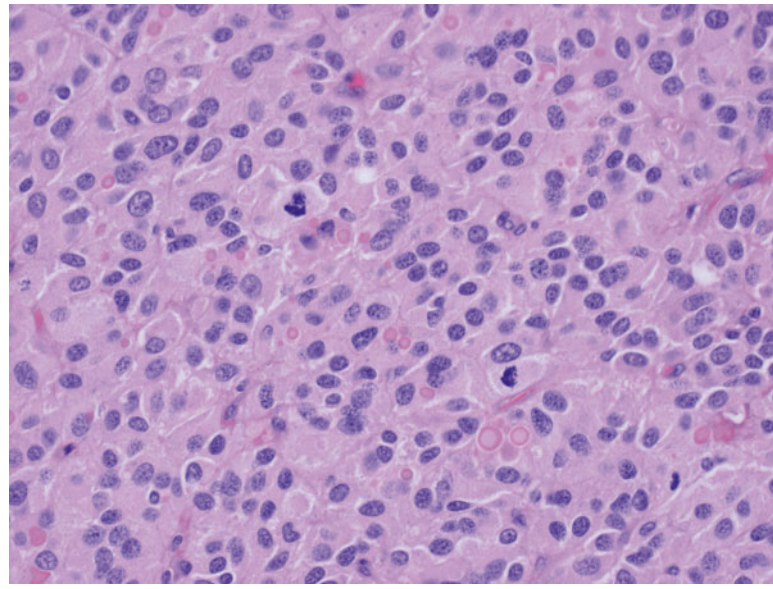

Fig. 15.33 This steroid cell tumor has cells with eosinophilic cytoplasm. There are several mitotic figures – one of the features predictive of malignant behavior (H&E ×400 magnification)

in regression of virilizing features with reappearance on tumor recurrence.

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16 Pathology of Malignancies Metastatic to the Ovary and of Synchronous Ovarian and Endometrial Carcinoma

Naveena Singh

Abstract

Metastases to the ovary, particularly when mucinous, are treacherously difficult to distinguish from primary ovarian neoplasms. In routine clinical practice, this remains among the commonest and worst misdiagnoses in gynecological pathology and one that pathologists and clinicians alike can easily make. The error can have especially dire clinical consequences when metastasis from a silent extraovarian primary presents as an apparent low-stage ovarian carcinoma, but can equally be made, to the detriment of management, in an ovarian neoplasm with a known other primary.

Over the last three decades or so, a volume of literature has appeared emphasizing the features of primary and metastatic ovarian tumors for correct distinction. There are many general clinical, gross, and histological features that are helpful, apart from features specific to metastases from particular types and sites of primary tumor. Metastatic tumors tend to be bilateral, relatively small in size, and show surface involvement and vascular invasion, though exceptions occur. Some histological patterns, such as signet ring carcinoma, colloid carcinoma, and tumors associated with pseudomyxoma peritonei, are essentially exclusive to metastases. Specific tumor types are discussed individually in the chapter.

The vast majority of cases can be accurately diagnosed with due attention to morphological features, with or without the help of immunohistochemistry, and it is only a tiny minority whose true nature may not become apparent till after a period of clinical follow-up.

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Pathology of Malignancies Metastatic to the Ovary

Introduction

It is well recognized that metastases to the ovary may be difficult to distinguish from primary ovarian neoplasms. This is particularly the case with mucinous neoplasms where the possibility of metastasis must be actively considered and excluded. The need for robust MDT correlation with all the clinical, radiological, and biochemical tumor marker information cannot be overemphasized in these situations. The pathological distinction of primary versus metastatic tumors to the ovary has been the subject of many historic and recent papers [1–9]. It is evident that a substantial proportion of tumors previously thought to be primary ovarian neoplasms are likely to have been metastatic, such as those associated with pseudomyxoma peritonei and widely disseminated mucinous carcinomas. Despite this increasing awareness among histopathologists, the distinction remains problematic in current clinical practice.

There are several reasons why this is a particular problem in ovarian pathology. Firstly, the ovary is a site of such a vast array of primary tumors that almost any type of metastatic tumor has an identical ovarian counterpart or a close mimic thereof. Secondly, the ovary, despite its small size, is a vascular organ and, particularly in reproductive life, appears to provide a good soil for metastasis for reasons that are incompletely understood; ovarian metastases are found in 30 % of women dying of cancer [10]. The consequences of this are that tumors metastatic to the ovary are not only common, comprising 6–7 % of all ovarian masses, but often large, dwarfing any manifestations of a primary elsewhere. Overall the incidence of discovering an ovarian metastasis prior to the primary tumor is 1 %, but this varies with primary site; while this may occur in around 1.5 % of breast tumors [11], around 10 % of colorectal metastases [3] and 60–70 % of Krukenberg tumors arise from hitherto undiscovered primaries [12]. Thirdly, metastatic tumors growing within the ovary are often cystic, irrespective of the nature of the primary, exaggerating both the difference from the primary tumor and the similarity to an ovarian neoplasm.

Certain histological appearances complicate interpretation. Ovarian metastases are notorious for demonstrating the phenomenon of "maturation" whereby the metastatic tumor appears better differentiated in part or whole than the primary, making it more difficult to relate the ovarian mass to the primary and also increasing the resemblance to a primary ovarian tumor, where background mimics of benign or borderline elements may be seen. Metastatic solid tumors often demonstrate follicle-like spaces, resembling those seen in a variety of primaries. Stimulation of the background ovarian stroma also produces unique conundrums: the extreme stromal proliferation seen in some Krukenberg tumors may mask the presence of the metastatic carcinoma cells themselves. In addition, metastatic mucinous tumors commonly elicit hormonal manifestations by inducing stromal luteinization leading to sex hormone production and causing clinical and morphological suspicion of a primary neoplasm [13].

The distinction of a metastatic tumor from a primary ovarian neoplasm is paramount as it will influence subsequent patient management. This is of particular importance in certain circumstances.

- In centers where frozen sections are carried out, a pathologist may be asked to make this distinction in order to decide whether to proceed to cytoreductive surgery and staging and occasionally prior to catheter placement for intraperitoneal chemotherapy.
- In certain situations, as when the patient is too ill for major surgery or has a history of a previous malignancy, it is imperative to make the correct diagnosis on a core biopsy so that appropriate treatment can be instituted.
- Accurate diagnosis is required for targeted therapy which may be in the context of a clinical trial.
- Accurate diagnosis enables the patient and her family to obtain correct prognostic information and appropriate counseling.

General Characteristics of Metastatic Ovarian Tumors

There are many general clinical, gross, and histological features that are helpful in this distinction, apart from the features specific to metastases from particular types of primary neoplasms detailed below. These features are discussed below and summarized in Table 16.1. Immunohistochemistry as an adjunct has a definite role in the distinction of a primary ovarian carcinoma from a metastatic tumor; the immunophenotype of mucinous ovarian tumors is summarized in Table 16.2 [14–18]. Most cases, however, can be confidently diagnosed on routine H&E preparations, with adequate clinical history including all the information obtained at a gynecological oncology MDT. A minority of cases remain after thorough workup, further sampling, immunohistochemistry, and, when indicated, clinical investigation that are labeled as carcinoma of unknown primary site [2, 14].

Clinical Features

The diagnostic process should begin before pathological examination. As mentioned above, robust MDT discussion, including a history of previous malignancies, is imperative and if present should prompt careful consideration of the possibility of ovarian metastasis. Metastases to the ovary can arise from any body site, but most commonly these are from the large bowel, elsewhere in the female genital tract, stomach, pancreaticobiliary tract, and breast. Of the various primary sites, those most likely to masquerade as primary ovarian tumors of mucinous type are gastrointestinal, pancreatic or biliary tract, and endocervical adenocarcinomas, while tumors with an endometrioid appearance may represent colorectal metastases. In the presence of an endometrial primary, the diagnosis of metastasis is complicated by distinction from independent endometrioid tumors developing synchronously at both sites; this topic is dealt with separately later in this chapter. With mucinous tumors, the extent of disease is also of value. With rare though significant exceptions, the majority of primary mucinous ovarian carcinomas are usually FIGO stage I or II at presentation, and in broad terms, a widely metastatic mucinous carcinoma is far more likely to be a non-ovarian primary. With very rare exceptions of examples arising in association with teratomas, ovarian tumors in the syndrome of pseudomyxoma peritonei are metastatic, usually from the appendix. Other clinical features which may be helpful are symptoms related to the primary tumor rather than the ovarian mass, such as abdominal pain, rectal bleeding, dyspeptic symptoms, or jaundice. It should be noted that a raised serum CA125 is nonspecific and may occur in significant numbers of cases of metastatic ovarian tumors. Similarly hormonal manifestations, although common in primary ovarian neoplasms, can occur with metastatic tumors.

Gross Features

Laterality: Bilaterality is a strong pointer to metastasis in ovarian carcinomas other than those of serous morphology (Fig. 16.1). Primary endometrioid and mucinous carcinomas are rarely bilateral and if so should prompt consideration of the possibility of metastasis. It should be noted though that metastatic tumors are not invariably bilateral – around 70 % of all metastases are bilateral (WHO) (i.e., 30 % are not), and, conversely, 10 % of bilateral ovarian masses are metastatic tumors [5].

Tumor size: In general, primary mucinous carcinomas are larger than metastatic mucinous carcinomas. One of the widely held reasons for this is the apparent origin of primary mucinous carcinomas from benign and borderline mucinous neoplasms which tend to be the largest ovarian masses overall. Metastatic tumors are usually smaller though they may attain large sizes, and this feature on its own should be regarded as having "soft" significance [2, 5].

Size and laterality: Algorithms have been proposed combining size and laterality in predicting the primary versus metastatic nature of mucinous ovarian masses, particularly at frozen section. These classify bilateral tumors as metastatic, with or without an added size criterion of <10 cm favoring metastasis and > or = 10 cm favoring a primary [5]. This correctly distinguished 84 %

Table 16.1 Features useful in distinction of metastatic and primary ovarian tumors

Feature	Primary	Metastatic	Comment
Laterality	Unilateral	Bilateral	Enlargement in bilateral metastases may not be symmetrical, thereby appearing unilateral on imaging and macroscopic appearance (e.g., in signet ring carcinoma)
Size	Maximum tumor diameter >12 cm	Maximum diameter <10 cm	About 15 % of metastatic tumors will not be correctly assigned on size and laterality criteria; cutoff points of 12 and 13 cm have been published, offering marginally better prediction; it has also been suggested that unilateral tumors 10–15 cm should be considered indeterminate
Extensive intra-abdominal spread (mucinous tumors)	Unlikely in primary ovarian mucinous carcinoma	More likely to be metastatic than primary	An alternative primary site may not be found in all such cases; rare true disseminated ovarian mucinous primaries have been reported
Multinodular growth pattern with intervening normal parenchyma	Not usual	Characteristic	
Surface involvement	Not usual, except in background endometriosis and tumors arising thereof	Characteristic	
Hilar involvement	Absent/not typical	Typical in tumors that have metastasized through hematogenously	
Patterns specifically favoring primary or metastatic carcinoma	Associated benign, borderline, and malignant appearing areas*; complex papillary architecture; association with background changes: endometriosis (seromucinous and endometrioid tumors), Brenner tumor, mature cystic teratoma, Sertoli–Leydig cell tumor, adenofibroma	Signet ring carcinoma; pseudomyxoma peritonei (ovarii); colloid carcinoma; infiltrative pattern of small glands with desmoplastic reaction, single-cell infiltration	*Maturation of ovarian metastases may result in a deceptively similar gradation of features
Extensive vascular invasion	Not usual	Favors metastasis	

of all tumors in two studies [4, 19]), but can be refined by increasing the size cutoff to 12 or 13 cm, the last correctly categorizing 98 % of primary tumors, 82 % of metastases, and 87 % overall [19]. It should be remembered, however, that 10–15 % of tumors will be incorrectly categorized using this approach alone, and all clinicopathological parameters should be carefully evaluated. The most common outliers to a size and laterality algorithm are metastatic colorectal carcinoma, which continues to be diagnostically challenging to the pathologist, and metastatic endocervical carcinoma. When these two diagnoses are in the differential diagnosis, greater vigilance is required when applying this algorithm [19]. Fortunately, as detailed later in the section on individual entities, these are the two primary sites most easy to identify with the help of immunohistochemistry. Pancreatic metastasis may also be problematic, and the use of the algorithm

Table 16.2 Immunohistochemistry in mucinous tumors of the ovary [14–18]

Marker	CK7	CK20	CEA	CA19.9	CDX2	CA125	ER	DPC4/SMAD4	P16	PAX8	Beta-catenin
Ovary, intestinal type	Diffuse, may be focal	Focal, rarely diffuse	Focal or diffuse	Diffuse	Focal	Negative	Negative	Diffuse	Negative or focal	Usually negative	Sometimes positive
Ovary, Mullerian type	Diffuse	Negative	Negative	Negative or focal	Negative	Diffuse	Diffuse	Diffuse	Negative or focal	Positive, usually diffuse nuclear	Sometimes positive
Colorectal	Negative (rectal cancers may be positive)	Diffuse	Diffuse	Diffuse	Diffuse	Negative	Negative	Diffuse	Negative or focal	Negative	Positive
Appendix	Negative, may be positive	Diffuse	Diffuse	Diffuse	Diffuse	Negative	Negative	Diffuse	Negative or focal	Negative	Usually positive, may be negative
Pancreas and biliary tract	Diffuse, may be focal	Negative, may be focal	Diffuse or focal	Diffuse	Focal	Negative	Negative	Negative in about 50 %	Negative or focal	Negative	Variable
Stomach	Diffuse, may be focal	Negative, may be focal	Diffuse or focal	Diffuse	Focal	Negative	Negative	Diffuse	Negative or focal	Negative	Positive
Cervix	Diffuse	Negative, may be focal	Diffuse or focal	Negative	Negative or focal	Diffuse	Negative or focal	Diffuse	Diffuse	Positive	Variable

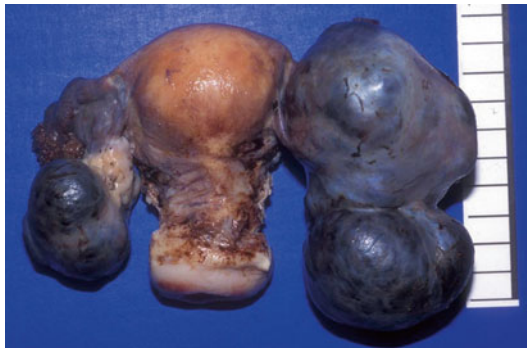

Fig. 16.1 Bilateral involvement of the ovaries is a pointer to metastasis (Courtesy of Dr Nafisa Wilkinson, St James's Hospital, Leeds, UK)

alone may be unhelpful in establishing a definitive diagnosis without adequate clinicopathological correlation.

Surface involvement: In addition to direct spread, tumors metastasize to the ovary via transcoelomic, transtubal, as well as blood-borne and lymphatic routes. Transcoelomic and transtubal spread typically result in surface deposits with characteristic microscopic features detailed below. Serous tumors also involve the surface and this parameter should be evaluated in the light of the histological subtype. Tumors arising via the blood or lymphatics do not involve the surface but manifest other histological features.

Gross features that are not discriminatory: It is important to note that several features on gross examination were found *not* to be of diagnostic value in distinguishing primary from metastatic carcinomas in one study [6] – a cystic gross appearance, the mucinous or non-mucinous appearance of cyst contents, the presence of solid or papillary areas, and the presence of hemorrhage or necrosis.

Histological Features

Bilaterality: While this feature is suggestive of metastasis in non-serous neoplasms, it may not be apparent on gross inspection as metastatic involvement can be disproportional within the two ovaries with histological examination required to confirm the presence of metastasis in an apparently "normal" ovary.

Surface involvement: Surface involvement too may not be conspicuous to the naked eye but only visible on histological examination. The microscopic appearance of the surface nodules, believed to arise from tumor cells directly implanting on the ovarian surface via transcoelomic spread or through the tubes, is characteristic. These often protrude over the ovarian capsular surface. They elicit a florid desmoplastic reaction, which may cause them to appear as depressed foci with surrounding fibrosis. Serous tumors and tumors associated with endometriosis may also involve the surface but do not elicit a desmoplastic reaction or the other features described below. The histological presence of mucin on the ovarian surface is also suggestive of metastasis [6].

Nodular growth pattern: A characteristic finding in metastatic tumors is a multinodular growth pattern within intervening areas of normal ovarian anatomy. Often the individual nodules vary not only in size but also in their histological and cytological content with single-cell infiltration, glandular areas, and cystic areas which may appear deceptively mature. Primarily emphasized in mucinous tumors, but equally applicable to non-mucinous tumors, a nodular growth pattern or a combination of features, described as "heterogeneous nodularity," is strongly suggestive of metastasis [2].

"Maturation": This is a phenomenon observed in metastatic mucinous tumors, whereby the epithelial cells display a range of appearances from highly atypical to bland, mirrored in architectural differences, with the more mature areas appearing more cystic. The appearance results in a dissimilar appearance from the tumor at the primary site and also an impression of carcinoma developing in the background of a benign or borderline tumor [1].

Infiltrative pattern: A widely variable morphological pattern of infiltration with histological features including small glands or tubules as well as single-cell infiltration favors metastasis. Primary mucinous carcinomas with foci of destructive stromal invasion may occasionally show some of these features, but the overall infiltrative pattern is not usually as heterogeneous as that encountered in metastases.

Vascular invasion: This is a distinctly uncommon feature in primary ovarian neoplasms but is found in carcinomas metastatic to the ovary. Involvement of the ovarian hilum and, in particular, hilar vessels is also a feature of blood-borne metastases.

Histological patterns almost exclusively associated with metastasis: Some histological subtypes are almost diagnostic of secondary involvement, namely, signet ring carcinoma, colloid carcinoma, and ovarian tumors associated with pseudomyxoma peritonei. Other than in exceptionally rare cases, usually in association with teratomas and, in the case of signet ring carcinoma, a few unusual mimics, these patterns are virtually diagnostic of metastasis, and relevant investigations should be encouraged.

Histological findings favoring a primary tumor: It is worth listing at this point some general features that favor a diagnosis of a primary tumor. Although these findings are predominantly relevant to mucinous tumors, they are of some utility in other subtypes. Primary mucinous tumors tend to have an expansile invasive pattern in which the neoplastic glands are arranged back to back with no intervening stroma [20]. Other patterns favoring primary tumors are a complex papillary pattern, microscopic cystic glands, and necrotic luminal debris [6] and the presence of mural nodules which are solid areas of a cyst wall that may contain anaplastic carcinoma or sarcomatous components. Background changes that favor origin of the tumor within the ovary are the presence of endometriosis, a mature cystic teratoma, an adenofibroma, a Brenner tumor, or a Sertoli–Leydig cell tumor. In many studies, the presence of benign-appearing and borderline-appearing areas has also been stated to favor primary tumors; but this feature requires cautious distinction from the phenomenon of maturation seen in some many metastases to the ovary.

Histological findings that do not help in the distinction of primary and metastatic tumors: Finally, it is useful to list features that are of no diagnostic value as they may be seen in both primary tumors and metastases. These are the presence of stromal mucin resulting in a pattern described as "pseudomyxoma ovarii," cribriform, villous, or solid growth patterns; focal areas resembling typical colonic carcinoma; and the presence of goblet cells. Tumor grade also does not distinguish between primary and metastatic tumors [6].

Specific Features of Metastases from Different Sites

Krukenberg Tumors

Definition

This classical type of ovarian metastasis is described first. The term Krukenberg tumor should be reserved for tumors showing its classical morphology: these are carcinomas composed of an appreciable component of signet ring cells, arbitrarily defined as occupying >10 % of the tumor [2]. The presence of a prominent stromal component as described in historic papers, although typical, is not considered necessary, as this component is highly variable and its presence and amount are clinically irrelevant. Other tumors composed of cells that may show signet ring morphology, principally clear cell carcinoma and goblet cell carcinoid, should be excluded. The term Krukenberg tumor is often loosely used to describe any metastatic tumor, but this term should be reserved for metastatic signet ring cell carcinoma. Tumors with these features should be regarded as metastatic in every instance. In two-thirds of cases, the primary tumor is not clinically manifest and may be difficult to detect even when specifically sought [12]. Cases of so-called primary Krukenberg tumor are vanishingly rare, and this should be a diagnosis of exclusion [21].

Clinical Features

Krukenberg tumors most commonly metastasize from the stomach. Their incidence in the ovary depends on the incidence of gastric carcinoma, and these are most common in Japan. Gastric signet ring cell carcinoma is reported to be four times more likely to metastasize to the ovary than any other cancer in the body. This may be because of its high propensity for vascular spread and the fact that these tumors occur in younger women when the ovary is more vascular. The average age

of patients is 45. Clinically these manifest with signs of an ovarian mass or, especially in pregnant women, with hormonal manifestations due to stromal luteinization and hormone production; often the hormonal manifestations are androgenic. In other cases presentation may be due to metastasis to sites other than the ovary. Notably in approximately 30 % of cases, the patient does not have obvious signs or symptoms relating to the primary tumor. In terms of origin three-fourths of Krukenberg tumors originate from gastric primaries. The remainder arise from the large bowel (11 %), breast (4 %), appendix (3 %), biliary tract (3 %), and rarely other sites. These tumors have a poor prognosis [22].

Pathology

Grossly, these tumors are typically bilateral, solid masses with a smooth but bosselated surface. About 80 % of cases are macroscopically bilateral while in the remainder the involvement of the apparently normal ovary may only be detected on histological examination; a factor that can be misleading during frozen section evaluation. The average size is 10 cm. The cut surface varies with the relative amounts of extracellular mucin and stromal proliferation. Typically, the cut surface is solid, firm, and white, but nodularity, cystic change, a soft consistency, and a yellow appearance due to luteinization may occur. Often the periphery of the tumor is firm and dense producing a peripheral rind with a softer center.

Signet ring cells have a characteristic appearance in that they are round or polygonal cells with an eccentric nucleus that is compressed to the periphery by a large pale mucin-filled vacuole (Fig. 16.2). Although most cases show cells with these characteristic appearances, there are variations. Sometimes the cytoplasm is not pale but densely eosinophilic (Fig. 16.3). In other instances, the vacuole contains a targetoid inclusion (Fig. 16.4). In some cells the nuclei may be central rather than eccentric, resulting in a strong resemblance to clear cell carcinoma. There are usually large numbers of cells which have very little or no mucin. The tumor cells are in solid sheets but may appear nested or seen in strands or singly separated by stroma.

It is important to note that although a diffuse pattern of signet ring cells is pathognomonic, it is seldom seen in pure form. A degree of gland formation is often present (Fig. 16.5). This is in the form of small- and medium-sized tight tubules lined by cuboidal or flattened cells (Fig. 16.6). Occasionally, larger glands and cysts may also be seen (Fig. 16.7). It is also not uncommon to see nests of tumor cells containing goblet cells, identical to those seen in goblet cell carcinoids

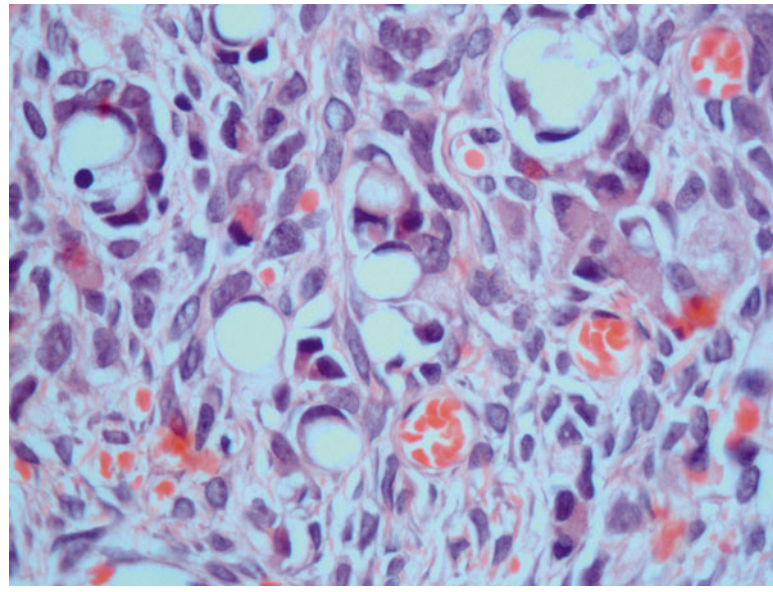

Fig. 16.2 The characteristic appearance of signet ring cells: these are round with an eccentric nucleus that is compressed to the periphery by a large pale mucin-filled vacuole

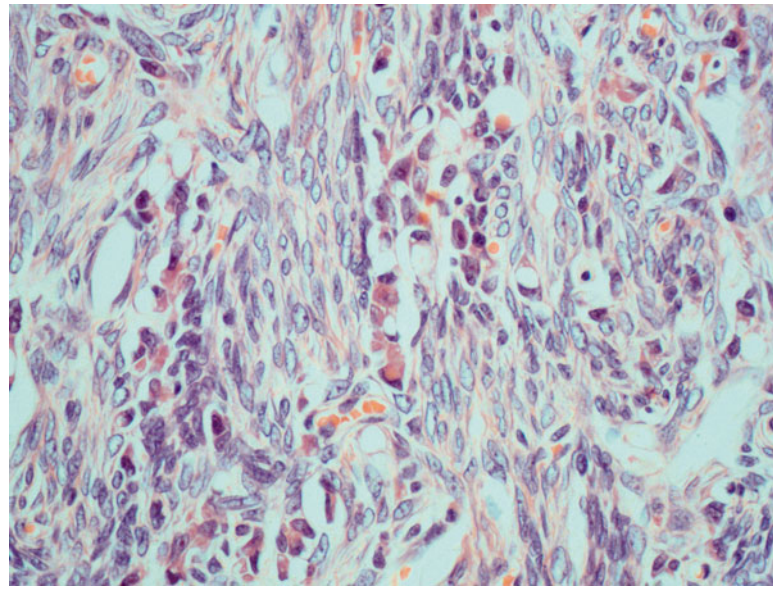

Fig. 16.3 Sometimes cells in signet ring carcinoma may show densely eosinophilic cytoplasm

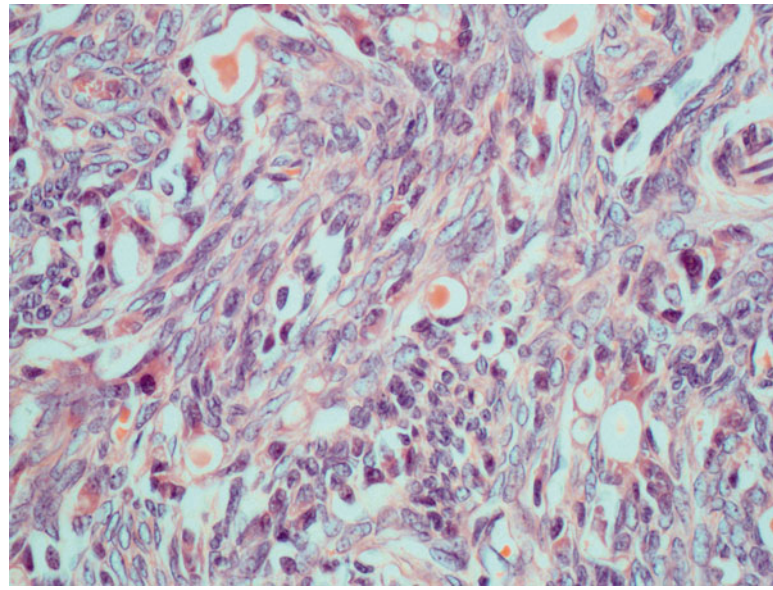

Fig. 16.4 Signet ring cell with eosinophilic targetoid inclusion

of the appendix. These are usually negative for neuroendocrine markers or may show focal positivity which does not preclude the diagnosis of Krukenberg tumor.

A characteristic feature of Krukenberg tumors is the stromal proliferation. The frequent prominence of this component led to its first description as "*fibrosarcoma ovarii mucocellulare* (mucinous sarcoma)" whereby the stroma was considered to be one component of a biphasic tumor. It was soon recognized that the stroma is reactive and very variable in amount and histological pattern. The stroma may be markedly prominent to the point of almost obscuring the single pale tumor cells; this is a known diagnostic pitfall (Fig. 16.8). In such cases, the stroma is cellular and shows a storiform arrangement of cells which are not atypical, resembling cellular fibroma. The combination of a cellular stroma with bland-appearing glandular structures may

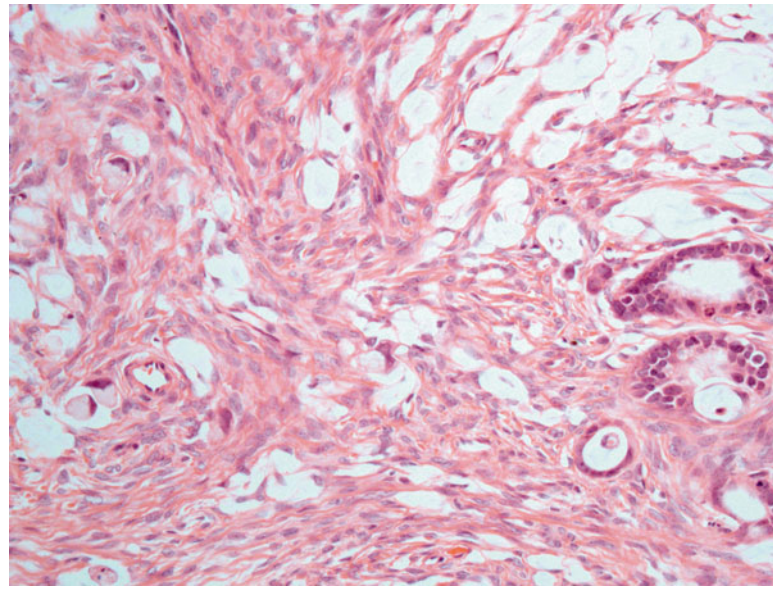

Fig. 16.5 Krukenberg tumor – it is common for the diffuse pattern of signet ring cells to be accompanied by glands and tubules; in some tumors the majority of the architecture may in fact be glandular

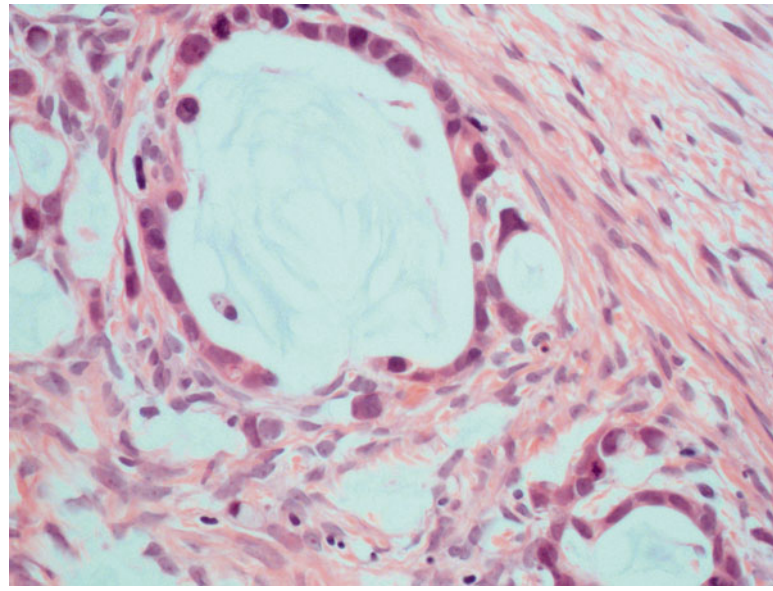

Fig. 16.6 Glands and tubules in Krukenberg tumors are often lined by a flattened, cuboidal, and deceptively bland epithelium

result in a pattern resembling an adenofibroma. In other cases the stroma is less cellular and may be markedly edematous, with small pale groups of tumor cells at the periphery, another pitfall. Alternatively, it may be in the form of strands separating the tumor cells and producing a pseudolobular pattern. In some cases thin wispy strands of stroma enclose extravasated mucin or compressed mucin-filled tumor cells resulting in a "feathery" appearance (Fig. 16.9). Finally, the appearance of the stroma may vary from one area to another in the same tumor, and it is common for the peripheral subcapsular areas to be more fibrous and dense with the central areas being more cellular and rich in tumor cells. In some tumors the stroma is inconspicuous. Another feature of the stromal proliferation seen most commonly in pregnant women is the presence of luteinized stromal cells, singly or in aggregates.

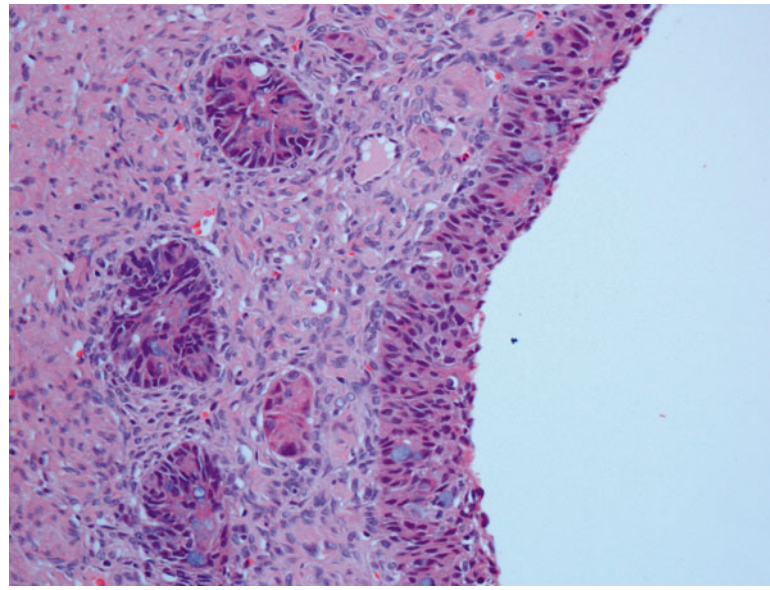

Fig. 16.7 Cystic areas are rarely present in signet ring carcinomas

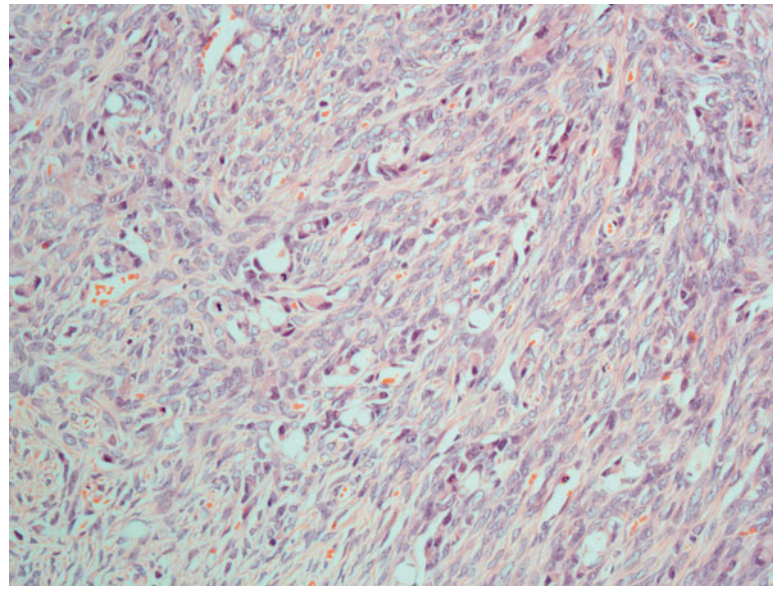

Fig. 16.8 Krukenberg tumor with markedly cellular stroma; this may sometimes obscure the scattered pale malignant cells

Other features are the common presence of lymphatic and vascular invasion, especially at the periphery of the tumor, the ovarian hilum, and at extraovarian sites such as the fallopian tubes and the uterus. Signet ring carcinoma is believed to metastasize hematogenously or by retrograde lymphatic spread, and surface involvement is not as common as in other metastases.

The diagnosis can be confirmed by positive staining for cytoplasmic mucin, using PAS after diastase digestion or mucicarmine. A panel of immunohistochemical markers should be used (see Table 16.2) although subclassification of the gastrointestinal primary tumor by immunohistochemistry is often unhelpful, and endoscopic examination together with an informed review by the radiologist at MDT can usually identify the site of primary origin. In general, gastric tumors are positive for CK7 in 55 % cases and CK20 in 70 %; therefore, dual marker profile expression is

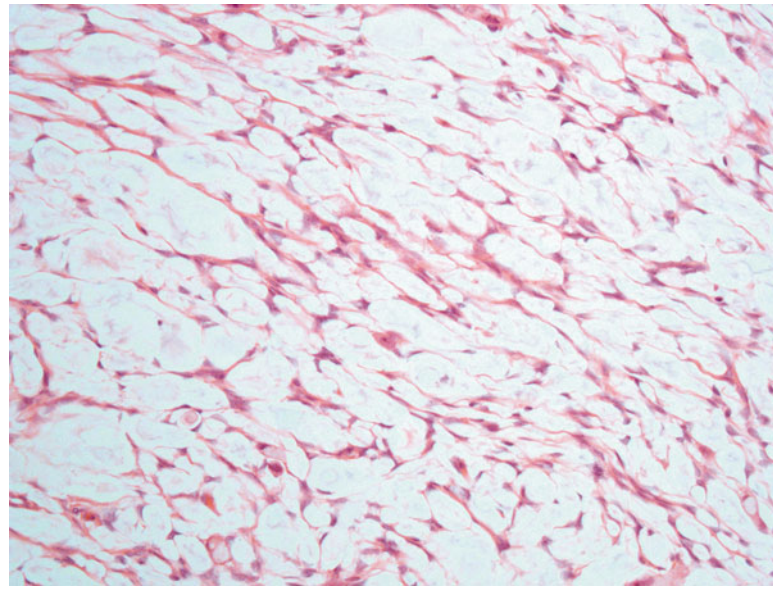

Fig. 16.9 Krukenberg tumor with thin wispy strands of stroma enclosing extravasated mucin or compressed mucin-filled tumor cells resulting in a "feathery" appearance

not uncommon (CK7+/CK20+). In addition they are CDX2 and Hep Par 1 positive and negative for estrogen receptor (ER). Tumors of colorectal origin are usually CK7−/CK20+ and also positive for CDX2, MUC2, and MUC5AC and negative for MUC1, Hep Par 1, and ER. Appendiceal tumors are usually CK7−/CK20+ or may show a CK7+/CK20+ profile and are otherwise similar to colorectal primaries. Tumors metastatic from the breast are positive for CK7, MUC1, and ER.

Differential Diagnosis

Clinicopathologically Krukenberg tumors can mimic sex cord–stromal, particularly Sertoli–Leydig cell tumors. This is because of the similar age incidence, frequent androgenic manifestations, and morphological overlap. Sertoli–Leydig cell tumors show a tubular component and, in case of heterologous differentiation, may have goblet cell containing glandular elements. Prominent stromal luteinization in Krukenberg tumors may be indistinguishable from a Leydig cell component. Furthermore, Sertoli–Leydig cell tumors can have cells resembling signet ring cells although these are invariably seen in association with goblet cell carcinoid-like neuroendocrine areas in cases with heterologous differentiation. The distinction between these tumors is by demonstration of a diffuse mucin-containing epithelial cell component and the greater atypia which may occur in Krukenberg tumors. Other non-epithelial mimics are sclerosing stromal tumors and signet ring stromal tumors; both of these can be distinguished by their other typical features and by the presence of positive lipid and negative mucin stains. Dysgerminoma also falls in the differential diagnosis of a solid tumor in a young woman, and this can on occasion show cells with eccentric nuclei resembling signet ring cells; however, these are negative for mucin stains and show other characteristic morphological and immunohistochemical features.

A few epithelial tumors may resemble Krukenberg tumor, most commonly clear cell carcinoma. Clear cell carcinomas can have signet ring cells, and conversely Krukenberg tumors may show cells with central rather than eccentric nuclei, resembling clear cells. Mucin stains are useful in distinction as the defining feature of Krukenberg tumors is intracytoplasmic mucin. Mucin may be seen in clear cell carcinoma; however, this is never intracytoplasmic, but seen within glandular lumina or over the cell surface. Clear cell carcinoma with cells resembling signet ring cells always shows areas with more typical morphology. Papillary architecture, seen frequently in clear cell carcinoma, never occurs in Krukenberg tumors. Serous, endometrioid, and

undifferentiated carcinomas may also show signet ring cells, but these are rarely frequent enough to cause a diagnostic problem. Rare examples of primary mucinous ovarian neoplasms containing signet ring cells have been reported, but the features of these differ from those of the usual Krukenberg tumor; it is recommended that these should be classified according to the underlying background neoplasm with a notation concerning the signet ring cell component, rather than being labeled as "primary Krukenberg tumors" which are exceedingly rare. Primary goblet cell carcinoids may show abundant signet ring cells and can be diagnosed by widespread neuroendocrine differentiation on immunohistochemistry. Tumors of mesothelial origin, including adenomatoid tumor and malignant mesothelioma, may show signet ring cells, but these are usually accompanied by other typical features, and epithelial mucin stains as well as immunohistochemistry for mesothelial markers can help in distinction.

Metastases from Colorectal Carcinoma Including Appendiceal [23] and Intestinal-Type Gastric Carcinoma [24]

Clinical Features

Tumors of colorectal origin top the list of ovarian metastases that can be mistaken for a primary ovarian tumor [25]. Overall ovarian metastasis occurs in about 7 % of colorectal cancers. In about half of these, the ovaries are involved as part of widespread peritoneal involvement while in the remainder the ovarian metastasis is the sole or predominant site of metastatic disease. A history of previous colorectal cancer is present in about 75 % cases; from this perspective one study has shown that an ovarian mass developing in a patient with a known history of colorectal cancer will turn out to be metastasis in 57 %, benign in 26 %, and a new primary ovarian malignancy in 17 % of cases [26]. In about 10 % of cases in the literature and in up to one-third of cases in some series [27], the ovarian mass may be the first clinical manifestation of a bowel cancer, and due vigilance by the pathologist is required to make the correct diagnosis. There are significant clinical differences between ovarian metastases developing in women with a known history of bowel carcinoma and a silent bowel primary. In comparison with women with metastasis from a known colorectal primary, those with no previous history have a greater likelihood of being significantly younger, presenting with symptoms and signs related to the ovarian mass and without any features related to the primary bowel tumor, having elevated CA125 levels, occasionally having large apparently unilateral tumors which may be mucinous, and show a CK7+/CK20+ immunophenotype [28]. In most cases the primary is discovered intraoperatively or following correct diagnosis of the ovarian mass, while in a small minority of cases, the colorectal tumor becomes apparent some months to years after the ovarian mass is removed.

There is a wide age range at presentation in the reported literature of 12–85 years [3, 27]. A few patients have hormonal manifestations such as abnormal uterine bleeding or breast tenderness, related to stromal luteinization. About 80 % of all metastases arise from primary tumors situated in the rectum or sigmoid, the remainder being within the descending colon, ascending colon, and cecum. Excluding tumors associated with pseudomyxoma peritonei, described below, metastases of appendiceal origin show broadly similar features to other intestinal tumors; it is important to note that the appendix in such cases is often firm and thickened but not grossly enlarged by a discrete mass and is therefore a classical silent primary [23]. A small number of intestinal metastases are from the small bowel. The routes of spread to the ovary are direct, transcoelomic, hematogenous, and retrograde lymphatic.

Pathology

On gross examination these tumors more often appear unilateral to the naked eye than Krukenberg tumors. They are large masses with an average size of 10 cm and may be ruptured or show surface involvement. These are solid necrotic masses with a minor cystic component and not mucinous in the majority of cases.

Histologically, metastases from the colon usually have an endometrioid appearance

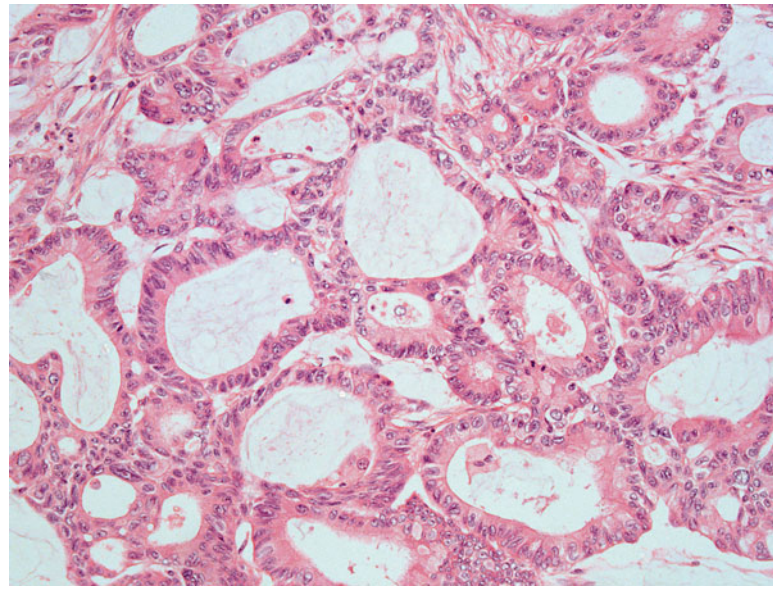

Fig. 16.10 Metastatic colorectal carcinoma often closely resembles primary endometrioid carcinoma

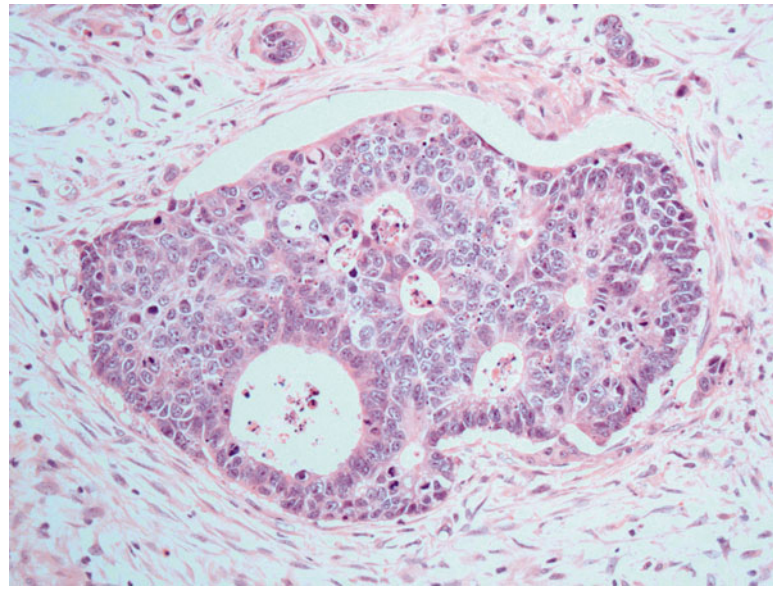

Fig. 16.11 On close inspection, despite the superficial resemblance to endometrioid carcinoma, metastatic colorectal carcinomas show a strikingly greater degree of nuclear atypia than would be seen in an endometrioid carcinoma of the corresponding architectural grade

(Fig. 16.10) [25]. Only a minority of cases are mucinous, rarely have a clear cell appearance, or may show a combination of these patterns. The endometrioid-like differentiation resembles endometrioid ovarian adenocarcinoma, with glands lined by stratified epithelium which does not show conspicuous mucin secretion. These tumors are composed of glands of varying sizes lined by non-mucinous columnar cells which show marked cytological atypia and frequent mitoses (Fig. 16.11). Two characteristic histological features have been described which must be applied judiciously as they may be seen occasionally in primary ovarian tumors. These are a "garland pattern" in which cystic glandular structures containing necrotic debris are surrounded by round tubular glands, arranged in a cribriform pattern, and "dirty necrosis" consisting of necrotic material with karyorrhectic debris (due to breakdown of carcinoma cells)

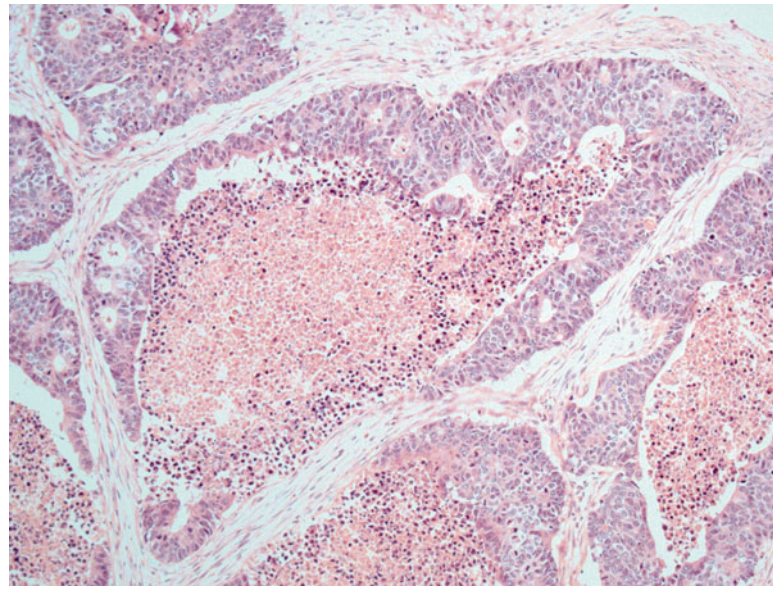

Fig. 16.12 Metastatic colorectal carcinoma exhibiting a "garland pattern" of cribriform glands with "dirty necrosis" consisting of necrotic material with karyorrhectic debris

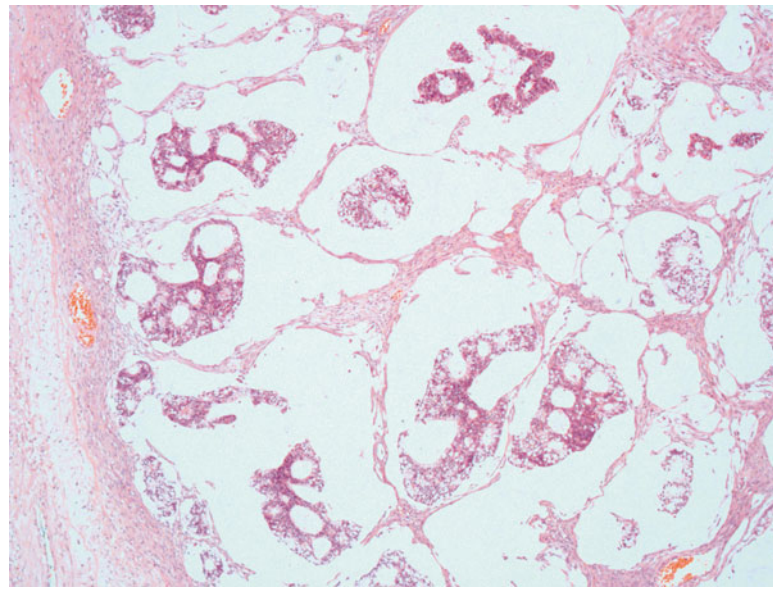

Fig. 16.13 Metastatic carcinoma with colloid carcinoma pattern characterized by malignant epithelium and glands within large pools of extracellular mucin

which can be seen within garlands and neoplastic glands (Fig. 16.12). In metastatic tumors with endometrioid differentiation, the absence of squamous metaplasia, adjacent endometriosis, or an adenofibroma may help make the distinction from primary endometrioid carcinomas. Also helpful are scattered cells with goblet cell morphology or cytoplasmic mucin secretion which are often present in metastatic colorectal carcinomas.

Some examples of colorectal metastases are frankly mucinous, with cystic and glandular structures lined by mucin-secreting epithelium, as with other mucinous metastases. There is one pattern of mucinous carcinoma which occurs almost exclusively in metastatic carcinoma, usually of colorectal origin: this is the so-called colloid carcinoma pattern. This is characterized by malignant epithelium and glands within large pools of extracellular mucin (Fig. 16.13). Such

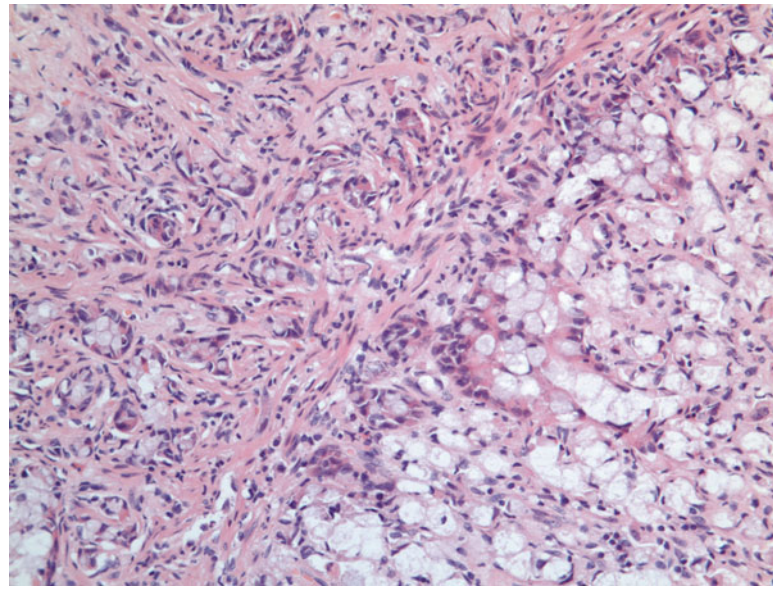

Fig. 16.14 Malignant metastatic goblet cell carcinoid (mixed adenoneuroendocrine carcinoma) in the ovary (Courtesy of Dr Nafisa Wilkinson, St James's Hospital, Leeds, UK)

a pattern is rare in primary mucinous ovarian tumors and metastasis, including one of appendiceal origin, and should be vigorously excluded.

Colorectal tumors can also present as classical Krukenberg tumors with signet ring cell morphology or a combination of signet and intestinal-type differentiation. Those of appendiceal origin may show prominent tubular differentiation, prompting a consideration of goblet cell carcinoid though it should be remembered that true appendiceal goblet cell carcinoids rarely metastasize to the ovary [3] (Fig. 16.14).

A small minority of colorectal tumors metastatic to the ovary may show clear cell morphology, resembling clear cell carcinoma or a secretory variant of endometrioid carcinoma. The presence of bilaterality, focal mucinous differentiation, and colloid-like secretion within gland lumina and the lack of characteristic features of clear cell and endometrioid carcinoma, as well as confirmatory immunohistochemistry, should help to establish the true nature of the tumor.

In all types of tumors, the stroma may be edematous or cellular, and stromal luteinization may be seen.

On immunohistochemistry, colorectal origin is easier to distinguish with immunohistochemistry showing CK7−/CK20+/CDX2+ pattern of reactivity in the majority of cases. It should be noted that while the majority of tumors show a CK7−/CK20+ immunophenotype, this is not specific for colorectal tumors and may be seen with gastric and other primaries as discussed above. The CK7−/CK20+ phenotype is dependent on tumor location and grade of the colorectal primary; while one study has reported this pattern to be more likely to be seen overall with left-sided and better-differentiated tumors [29], another study has reported that rectal tumors are CK7+ in 74 % cases [30]. Around 70 % of gastric carcinomas [29] and 50 % of appendiceal carcinomas [23] are CK7 positive. Other markers such as beta-catenin and P504S and many others have been reported to improve specificity but are not in routine laboratory usage [14, 31–33]. Differential expression of mucins, particularly MUC2 and MUC5AC, is also reported to be useful [34].

Differential Diagnosis

For reasons already emphasized, the most important differential is with primary epithelial ovarian carcinoma, particularly endometrioid. Primary endometrioid carcinomas are usually unilateral, unlike metastatic carcinomas. These arise in a background of endometriosis or may show an associated adenofibromatous component. The carcinoma often shows focal squamous metaplasia which is not a feature of colorectal

tumors. These three features, namely, endometriosis, adenofibroma, and squamous differentiation, have been stated to be a classical triad of confirmatory endometrioid differentiation which should prompt one to favor a primary over a metastasis. Apart from these associated changes, the most helpful feature is the greater atypia and mitotic activity seen in metastatic colorectal tumors; the glandular architecture undoubtedly bears a superficial and sometimes strong resemblance to endometrioid carcinoma, but the nuclear atypia and mitosis are often out of proportion to that usually expected in a low-grade (gland-forming) endometrioid carcinoma. This appearance should prompt a search for mucin secretion which may be subtle and focal.

Tumors of colorectal origin with an endometrioid pattern can be distinguished from ovarian endometrioid carcinomas using ER, CA125, CK7, CK20, CDX2, and CEA [14]. Colorectal tumors are generally diffusely and strongly positive for CK20 and negative for CK7; this pattern is highly specific but not diagnostic of colorectal origin [29]. These are positive for CEA and CDX2 and negative for ER and CA125. Endometrioid ovarian carcinomas on the other hand are diffusely positive for CK7, as well as ER and CA125 and negative for CK20 and CEA. CDX2 staining may be present in endometrioid carcinoma, and it is useful to note that squamous morules are often positive. Staining for CEA should be interpreted carefully in tumor cells as necrotic debris and inflammatory cells may be positive.

Immunohistochemistry is less helpful in metastatic colorectal tumors with mucinous morphology as these show much greater overlap with primary ovarian tumors which usually have an enteric phenotype (see Table 16.2). Primary mucinous intestinal-type ovarian tumors are usually diffusely positive for CA19-9 and may be focally or less often diffusely positive for CEA, CDX2, and CK20; alternatively, they may be negative for these markers. CK7 staining tends to be *diffusely* positive in ovarian primaries and this is a useful discriminant, as most colorectal tumors are negative or at most focally positive, although this is dependent on the location and differentiation of the primary tumor [14, 29]. It should be remembered that a proportion of colorectal metastases may show a CK7+/CK20+ profile, and rare examples may be negative for both markers. Furthermore, 19 % of gastric carcinomas may be CK7−/CK20+ in common with tumors from other locations, such as the biliary tract. Mucinous tumors with a colloid carcinoma pattern are almost always metastatic.

The distinction of the rare metastatic colorectal carcinomas with a clear cell pattern has been mentioned above.

Mucinous Ovarian Tumors in the Presence of Pseudomyxoma Peritonei

Definition and Clinical Features

Pseudomyxoma peritonei (PMP) is a clinical term describing the presence of abundant mucoid or gelatinous material within the pelvis and abdominal cavity surrounded by fibrous tissue [10], which is caused by rupture, leakage, or metastasis of a mucinous neoplasm within the abdomen. It is now well accepted that this is almost always of appendiceal origin with secondary ovarian involvement and does not occur with primary ovarian mucinous neoplasms with the exception of rare cases in association with teratomas [8, 35].

Pathology

The appendiceal tumor is typically a low-grade mucinous neoplasm which may not show obvious invasion (Figs. 16.15 and 16.16). There are associated bilateral or right-sided large multicystic ovarian mucinous tumors, which develop secondarily after incorporation of mucin and mucinous epithelium from the cortical surfaces into the ovarian parenchyma. The presence of mucin over the ovarian capsular surface is characteristic. On cut section the ovaries resemble bags of viscid mucin.

Histologically the cysts are lined by very tall columnar cells bulging with mucin which leaks out of the luminal surface of the cells (Fig. 16.17). The cells have a bland or only mildly atypical appearance. Dissection of mucin into the ovarian stroma results in *pseudomyxoma ovarii* (Fig. 16.18); while it is

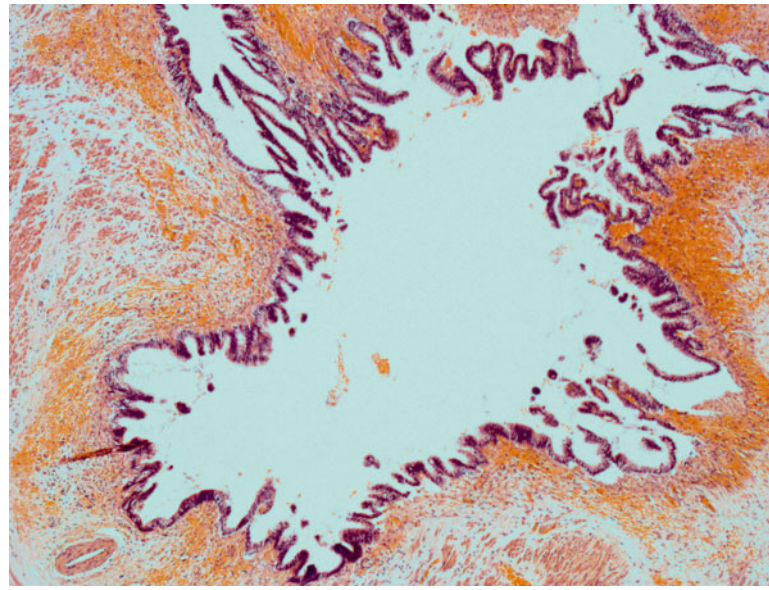

Fig. 16.15 The appendiceal tumor in cases of pseudomyxoma peritonei is typically a low-grade mucinous neoplasm which may not show obvious invasion

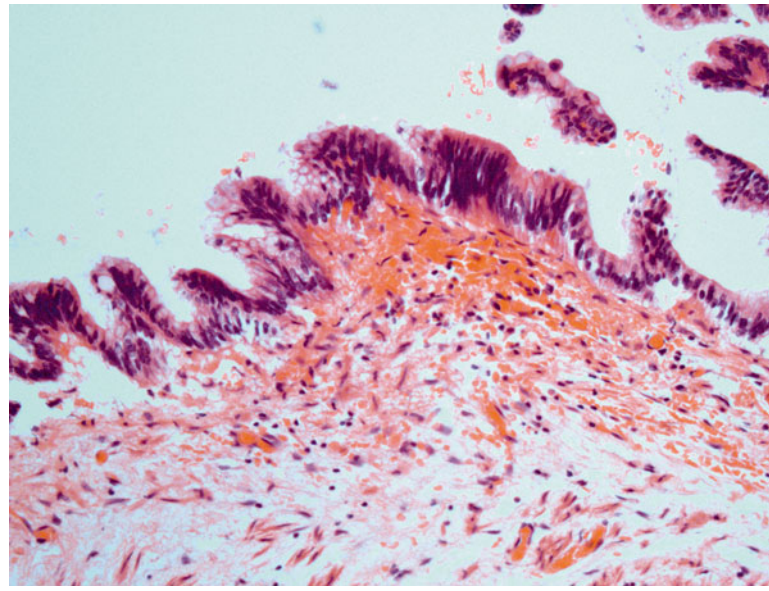

Fig. 16.16 Lining epithelium of low-grade appendiceal mucinous neoplasm shows relatively bland nuclear features

generally felt that this feature should strongly suggest metastasis, a study comparing numerous histological features in primary and metastatic mucinous ovarian carcinomas found it to be of no value in this distinction [6]. There is a variable amount of stroma between the cysts. The presence of mucin on the capsular surface may be confirmed histologically and it may or may not be associated with a desmoplastic reaction (Fig. 16.19).

Differential Diagnosis

Distinction from a primary ovarian neoplasm may be necessary. Primary ovarian mucinous neoplasms may cause mucinous ascites or PMP; these always occur in the context of mature cystic teratomas. Accurate diagnosis requires removal and histological examination of the entire appendix. This may show features of a mucocele, a frank neoplasm, or, in some instances, appear completely normal to the naked eye. Like the

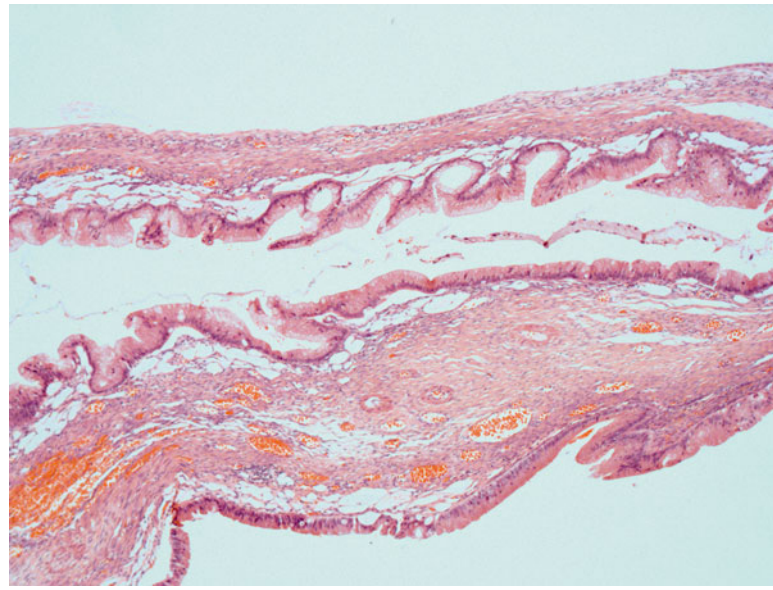

Fig. 16.17 Ovarian tumors in pseudomyxoma peritonei show mucin-filled cysts lined by very tall columnar cells bulging with mucin

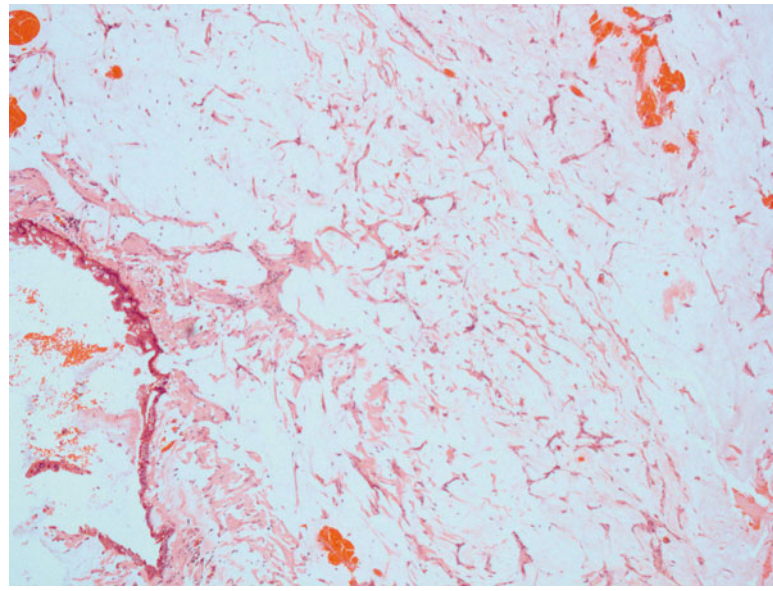

Fig. 16.18 Mucin extravasation in pseudomyxoma peritonei

tumor in the ovary, the appendiceal tumor is low grade and is composed of tall columnar cells bulging with mucin. There may be frank infiltration of the wall of the appendix with mucin on the serosal surface, but some cases do not show these features; it is postulated that sites of previous rupture may have been sealed off by fibrosis in these cases [36, 37].

Tumors associated with PMP may be metastatic from other sites, usually the large bowel. These tend to show greater atypia than those arising from the appendix.

Metastatic Tumors of Pancreatic and Biliary Tract Origin

Clinical Features

Tumors of the pancreas may metastasize to the ovary; an autopsy series showed this to be the third commonest site of primary disease following gastric and breast carcinoma [38]. In clinical

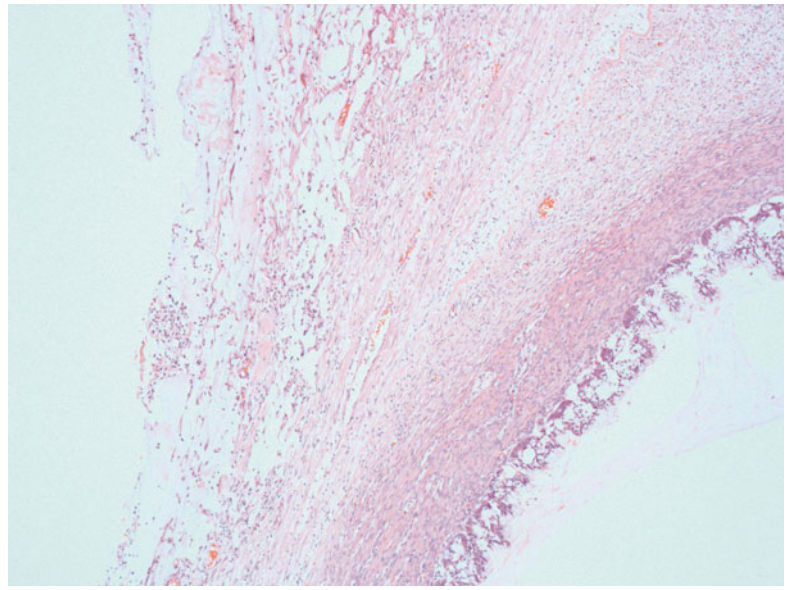

Fig. 16.19 Surface mucin in pseudomyxoma peritonei or other forms of metastatic mucinous carcinoma may be seen histologically, applied to the surface with or without a desmoplastic reaction, even when not macroscopically obvious

practice, these account for 7 % of nongenital tract and 19 % of tumors of gastrointestinal tract origin metastasizing to the ovary. Most of the metastases are from pancreatic ductal carcinomas, with other tumor types more rarely encountered. Metastases from the gall bladder and extrahepatic biliary tree are generally considered rare, although accounting for a significant number of cases in countries such as Thailand where there is a higher incidence of cholangiocarcinoma. Pancreaticobiliary tumors can be difficult to distinguish from primary ovarian mucinous carcinomas owing to significant morphological and immunohistochemical overlap.

These have been encountered in women aged between 21 and 87 years with a mean age of 59 years. In 6 % of cases in a series of pancreaticobiliary tumors [39] and in as many as 31 % of a series composed solely of gallbladder and biliary tract metastases [40], the ovarian tumor was detected prior to the detection of the primary. In most cases the primary tumor was detected at the same time as that in the ovary. Where follow-up was available, the majority of patients had died of disease after a mean period of 9 months. The correct diagnosis is therefore of importance because of the vastly different clinical outcomes. The majority of primary mucinous ovarian neoplasms behave in a clinically benign fashion.

Pathology

About 90 % of cases are bilateral, although in a minority of cases involvement of the second ovary may be only identified on histological examination. These tumors show an average size of approximately 10 cm, but range from 2 to 21 cm, and may be solid, solid–cystic, or multicystic. Capsular surface involvement is seen in 40 % of cases and in 66 % on microscopic examination. Around 60 % of tumors show a multinodular growth pattern, either grossly or histologically.

On histological examination, the tumors are usually mucinous and show a variation in histological patterns. Areas resembling borderline tumor and/or a cystadenofibromatous pattern are seen in almost 70 % of cases. An infiltrative growth is seen at least focally in 80 % of cases (Fig. 16.20). Other patterns which may be encountered are an endometrioid-like pattern, a small gland pattern (especially in metastasis from intrahepatic cholangiocarcinoma (Fig. 16.21) [41]), colloid carcinoma, Krukenberg tumor, or undifferentiated carcinoma [3, 40, 41]. A papillary growth pattern closely simulating a primary Mullerian malignancy has also been present on rare occasion [40].

On immunohistochemistry the tumor cells are always positive for CK7 and coexpress CK20 in

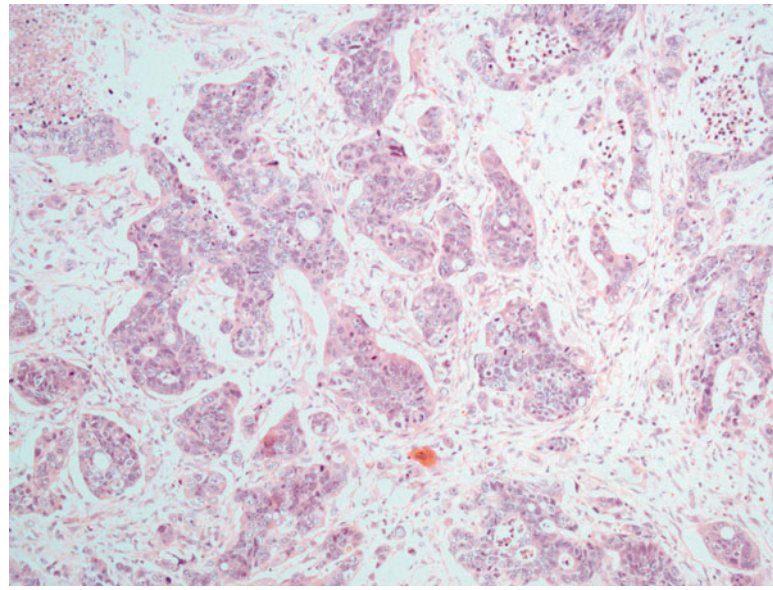

Fig. 16.20 An infiltrative growth pattern in a mucinous ovarian neoplasm, even if focal, is a strong pointer to metastasis

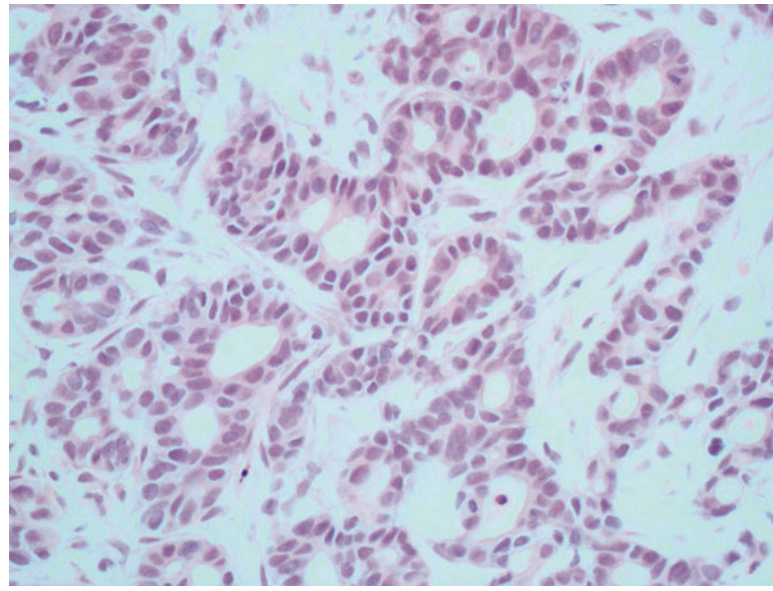

Fig. 16.21 A small gland pattern often seen with metastatic cholangiocarcinoma, though not exclusive to this source

about half of the cases. One of the most useful markers is loss of DPC4 expression [42] which occurs in 61 % of metastatic pancreaticobiliary carcinomas [39].

Differential Diagnosis

As has been emphasized in the introductory section and summarized in Table 16.1, distinction from primary ovarian mucinous tumors can be very difficult. Bilaterality, a nodular growth pattern, surface involvement, and infiltrative areas are all singly or in combination strongly suggestive of metastasis, and radiological or intraoperative exploration of a primary should be recommended. The distinction in individual cases can be difficult as the primary tumor may be clinically silent and presentation is with pelvic signs and symptoms due to the ovarian mass, ovarian involvement is unilateral in 10 % cases, and surface involvement may be absent. Almost

all reported cases show cystadenoma-like or borderline-appearing areas, and in 20 % of cases in the largest reported series, these were the sole patterns with no infiltrative growth seen [39]. On the other hand, severe nuclear atypia and intraepithelial carcinoma-like areas were seen in the vast majority, and peritoneal involvement was present in almost all cases reported. It is also widely reported that there is marked variation in morphology in different areas of the tumor and sometimes even on a single section, which should prompt the pathologist to exclude a metastasis. Immunohistochemistry is of limited value with the exceptions of CK17 positivity [43] and loss of expression of DPC4 which favor pancreaticobiliary origin; 55 % of pancreatic tumors and 10–50 % of biliary tract tumors show loss of DPC4 expression, while this is retained in 98 % of ovarian tumors [40].

Metastases from the Breast

Clinical Features

Metastases from breast carcinoma may be encountered in the ovary; these are not usually diagnostically problematic. After the gastrointestinal tract, the breast is the commonest source of nongenital tract ovarian metastases. This is reported in 10 % of cases in autopsy series and up to 50 % in therapeutic oophorectomies [3]. The incidence of metastatic breast carcinoma in risk-reducing salpingo-oophorectomies is very low at 1 % [44]. From a clinical perspective, an ovarian mass in a patient with a known history of breast carcinoma was found to be benign in 50 %, a new ovarian/tubal primary in 36 %, and metastatic breast carcinoma in 13 %; a malignant tumor in this scenario is therefore three times more likely to be a new primary than metastasis from the known breast cancer [45]. There are very rare reported instances of metastatic breast cancer presenting as an ovarian pathology prior to detection of the primary lesion [46]; in current clinical practice, this problem is virtually nonexistent.

Women with breast cancer metastatic to the ovaries were found to be more likely to be premenopausal with a genetic predisposition and suffering from tumors which are hormone-receptor positive, involving both breasts and showing lobular differentiation. The tumors were largely asymptomatic and discovered at a median interval of 5 years after diagnosis of the primary. Median survival was 3 years with significantly improved outcomes following optimal debulking surgery [47].

Pathology

Depending on the indication for oophorectomy, the ovaries may or may not be enlarged. There is often surface involvement, and this may appear papillary. The metastatic involvement has a multinodular appearance.

Histologically, lobular carcinoma has a greater propensity than ductal carcinoma to metastasize to the ovaries; in practice however most metastases are of ductal type (Fig. 16.22). The tumors may show a tubular/ductal/glandular pattern or show single-cell infiltration including a signet ring cell morphology. Rarely a papillary architecture may be encountered simulating an ovarian primary epithelial carcinoma. In such cases a review of the previous breast tumor and comparison with the metastasis to the ovary usually resolves the diagnostic dilemma.

Stromal luteinization is not a common feature. Vascular invasion is usually prominent.

Differential Diagnosis

Depending on the pattern, metastatic breast carcinomas may simulate a new high-grade ovarian primary, especially when detected after chemotherapy. A diffuse lobular pattern can resemble a granulosa cell tumor or small cell ovarian carcinoma. Tubular or insular patterns may simulate carcinoid tumors. Breast carcinomas at times can show a signet ring pattern reminiscent of a metastasis from the stomach, but this is usually not seen as a sole morphological pattern.

Immunohistochemistry is of value in distinguishing breast from ovarian carcinomas. A panel of WT1, CA125, and GCDFP is useful in this distinction [48], with breast tumors typically showing negative immunoreactivity for WT1 and CA125 and positive immunoreactivity for GCDFP. More recently, it is reported that the addition of PAX8 to this panel increases its

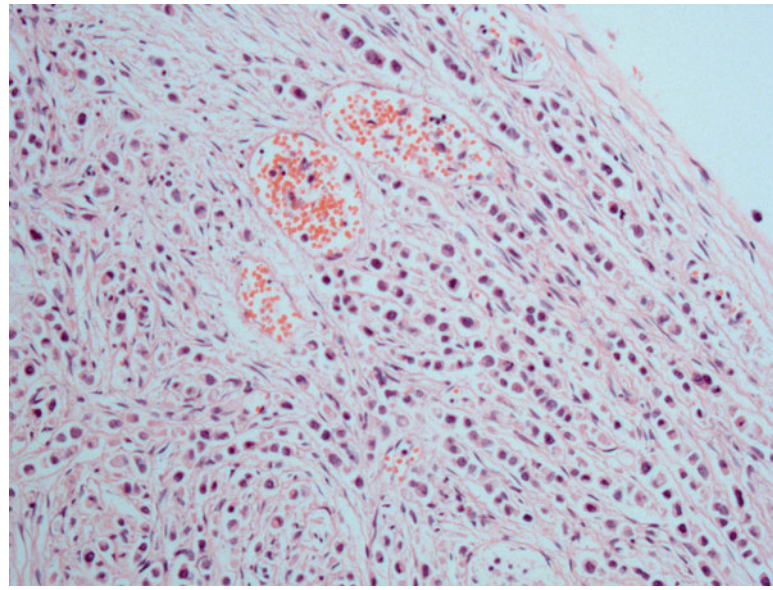

Fig. 16.22 Metastatic lobular carcinoma of the breast showing tumor cells in "Indian-file" arrangement

specificity as GCDFP is only positive in 43 % of breast carcinomas, and non-serous ovarian tumors do not express WT1 [18].

Metastases from Endometrial Carcinoma

Clinical Features

After the gastrointestinal tract, the endometrium is the commonest source of ovarian metastases, accounting for about 15–20 % of all cases. Spread may be transtubal, directly from serosal involvement or via lymphovascular invasion [49]. There are two problems in the differential diagnosis of ovarian metastases from endometrial carcinoma: the first is to distinguish a metastasis from an independent simultaneous primary tumor occurring in both the uterus and ovary; this is dealt with later in this chapter. The second is to distinguish high-grade uterine serous carcinoma from one of tubo-ovarian origin in cases involving both sites, as these are distinct entities in terms of their genetic abnormalities and response to chemotherapy [50].

For these reasons, it is difficult to describe the clinical features of metastatic endometrial carcinoma; these vary considerably with the type of the tumor. Synchronous tumors occur at a relatively younger age and have a more favorable prognosis than endometrial tumors metastatic to the ovary. In both groups clinical presentation is usually the result of signs related to the endometrial tumor, and it is unusual for an endometrial cancer to manifest first as an ovarian metastasis.

Pathology

Endometrioid tumors metastatic from an endometrial primary tend to share morphological characteristics, although the ovarian metastases tend to show a greater degree of nuclear atypia (Figs. 16.23, 16.24, and 16.25). Features useful in supporting metastatic spread, as opposed to an independent primary, are a higher grade and stage of the endometrial primary, including the presence of deep myometrial invasion, cervical stromal invasion, cornual involvement, or vascular invasion. The presence of surface involvement should be interpreted cautiously in this context as endometriosis often occurs over peritoneal surfaces and can be the source of a primary endometrioid ovarian adenocarcinoma; this feature therefore carries less weight in supporting metastasis than other features [3]. Immunohistochemistry is of no use in this regard as both metastatic and synchronous endometrioid tumors exhibit the same immunoreactivity.

Metastatic clear cell or serous carcinoma shows identical features in the endometrial and ovarian tumors. Uterine serous carcinomas

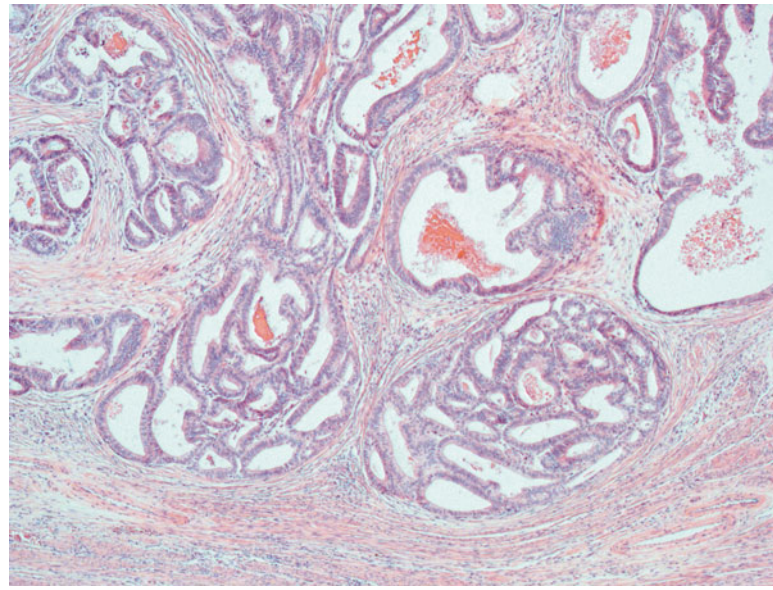

Fig. 16.23 Endometrial endometrioid carcinomas with ovarian metastasis usually show deep myometrial invasion

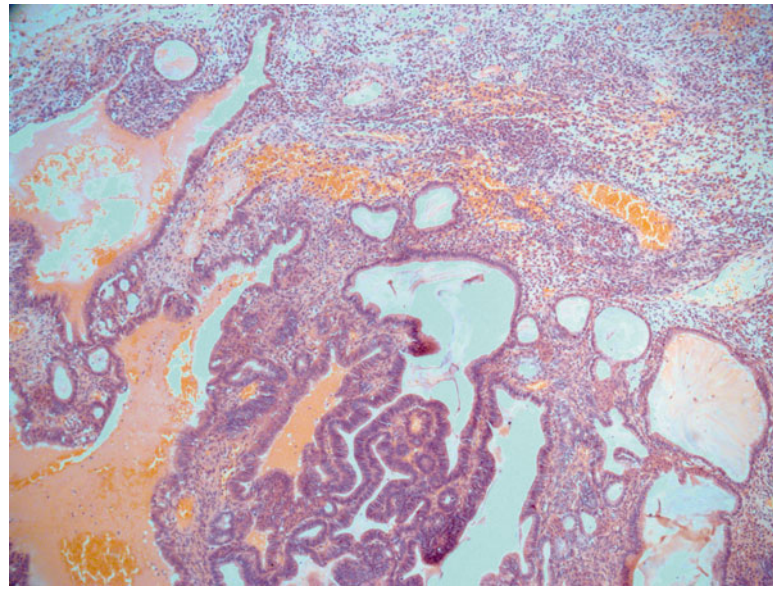

Fig. 16.24 Ovarian metastases from endometrioid endometrial carcinomas show similar morphology, but often appear to have greater nuclear atypia and may be associated with necrosis

occur in a background of an atrophic endometrium or an endometrial polyp. It is possible for these to metastasize widely, possibly by transtubal spread, without being deeply invasive at the primary site. The usual features of metastases apply when trying to establish if the tumor is primary or metastatic at this site. Ovarian involvement may be unilateral or bilateral, and surface involvement and vascular invasion are often present. In the presence of serous carcinoma involving both the endometrium and ovary, immunostaining for WT1 is valuable; this shows diffuse and strong positive immunoreactivity in tumors of tubal/ovarian origin, while there is negative or weakly and focally positive immunoreactivity in the vast majority of endometrial primaries [51]. The pattern of involvement and the site of bulky disease are invaluable in establishing the primary site of origin of the tumor.

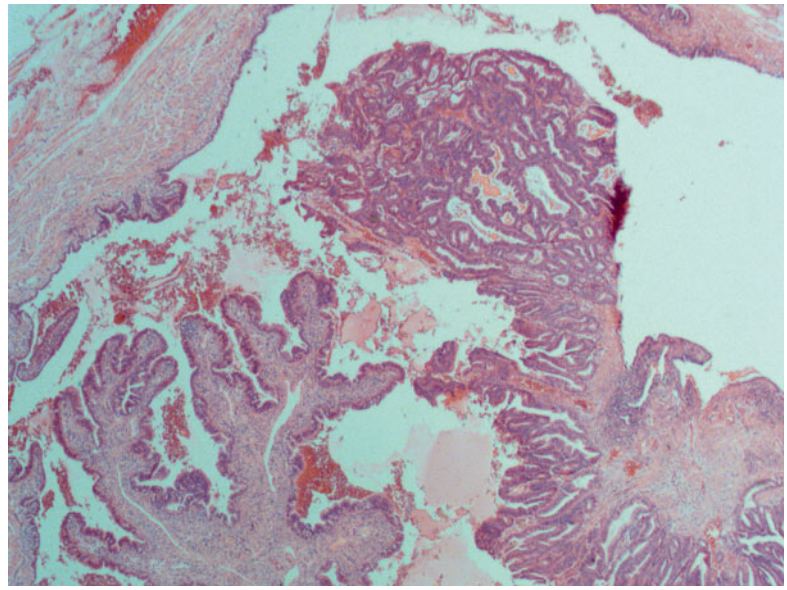

Fig. 16.25 In some instances ovarian metastases may be associated with carcinoma within the fallopian tube

Metastases from Cervical Carcinoma

Clinical Features

The cervix is the source of metastatic carcinoma to the ovary in a small number of cases. Adenocarcinomas are more likely than squamous carcinomas to metastasize to the ovary [52] and show some differences in clinical behavior as detailed below.

Carcinomas other than pure adenocarcinomas occur in young patients with a mean age of 43. In most cases the ovarian and cervical tumors are discovered simultaneously, but rarely the ovarian tumor may be the first manifestation of disease.

Metastases from endocervical adenocarcinomas may be difficult to recognize as such in both HPV-related and non-HPV-related cancers. In one large series, in 65 % cases, the cervical tumor was diagnosed concurrently or after the diagnosis of the ovarian mass, with the remaining minority of cases occurring in the context of a known history of cervical neoplasia. The knowledge of a previous cervical carcinoma does not always make the diagnosis easier because of confounding histological features that overlap with borderline ovarian tumors as described below.

Pathology

Excluding pure adenocarcinomas which are described below, cervical carcinomas metastatic to the ovaries were bilateral in 50 % cases and had an average size of 9 cm. The tumors were identical to the cervical primaries which were locally advanced and clinically evident. The histological types included squamous, adenosquamous, small cell, mixed small cell and adenocarcinoma, and undifferentiated carcinoma. As the morphological appearances of these tumors bear no resemblance to primary ovarian neoplasms, they are not diagnostically challenging to the pathologist [53]. In current practice, p16 immunoreactivity and testing for HPV by a variety of techniques can provide supportive evidence of the neoplasm being metastatic to the ovary as HPV is not found in primary ovarian tumors.

Metastatic cervical adenocarcinomas to the ovary are more problematic [54, 55]. These fall in the differential diagnostic category of metastatic mucinous carcinomas with all the difficulties described above. The simulation of primary ovarian tumors is greater than that seen with metastases from other sites due to their tendency to be large (mean size of 12 cm) and unilateral and to be detected prior to diagnosis of the cervical primary. In one series only 10 % of cases were bilateral with an infiltrative histological pattern. While the majority occur in association with an invasive cervical adenocarcinoma, in 11 of 29 cases (38 %) in this series the cervical

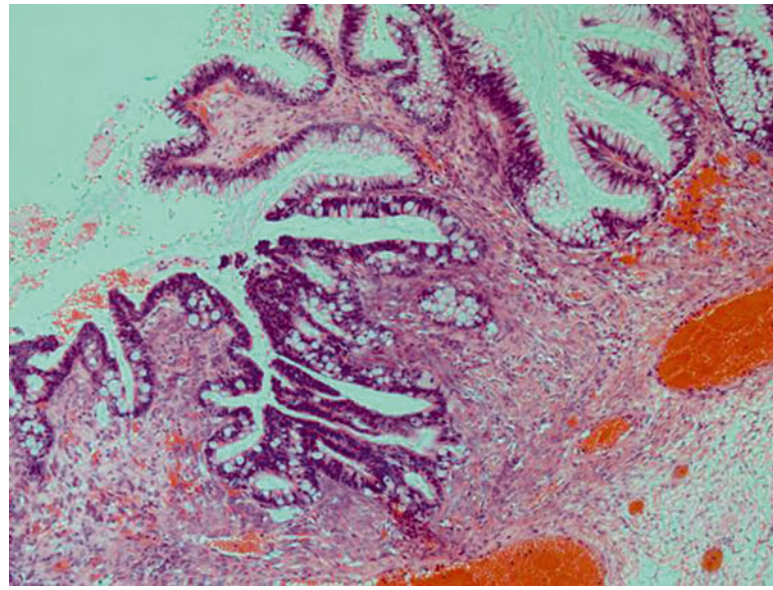

Fig. 16.26 Metastatic carcinoma from an endocervical mucinous primary may resemble a borderline tumor

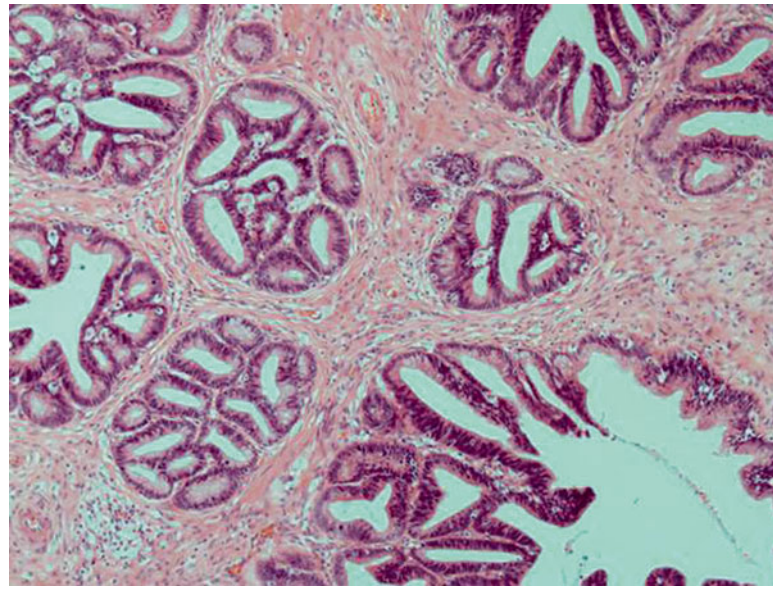

Fig. 16.27 The endocervical primary in these subtle cases often shows no or questionable invasion

primary was solely or predominantly composed of adenocarcinoma in situ with no definite invasive component. The histological patterns seen within the ovary include borderline-like confluent glandular, cribriform, and villoglandular areas similar to primary mucinous borderline tumors (Figs. 16.26, 16.27, and 16.28). Testing for HPV and immunoreactivity for p16 if diffuse and strong is valuable in HPV-related tumors [54].

Differential Diagnosis

Metastatic squamous cell carcinomas may on occasion require distinction from locally advanced tumors originating in teratomas or as a result of overgrowth of the malignant squamous component in an endometrioid carcinoma. The background elements of a mature teratoma would favor the former diagnosis while adjacent endometriosis would favor the latter.

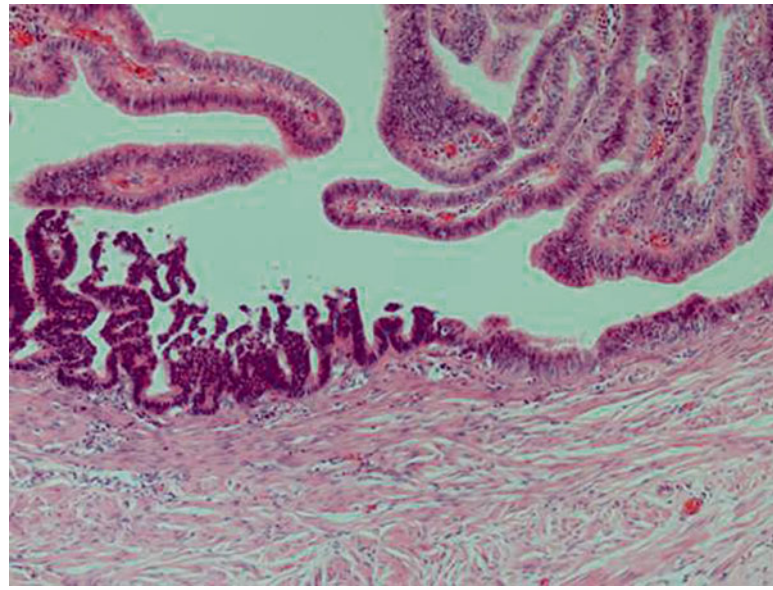

Fig. 16.28 The fallopian tube in the case illustrated in Figs. 16.25 and 16.26 showed foci of surface epithelial involvement

Metastatic mucinous tumors require distinction from primary ovarian neoplasms. This can be difficult even when there is known cervical neoplasia as this may be minimally invasive or entirely composed of adenocarcinoma in situ, and the metastasis may occur many years after treatment of the primary. It is postulated that such indolent cases may represent transtubal spread of the neoplastic cells as there is a frequent association with isthmic and endometrial involvement [55]. For HPV-related tumors p16 and HPV testing are of value.

Metastatic Malignant Melanoma

Clinical Features

Due to its protean morphological manifestations, metastasis from malignant melanoma can pose diagnostic problems at any site, and the ovary is no exception, particularly if the history of a skin or ocular melanoma is remote and/or not conveyed to the pathologist [3, 56–58]. These tend to occur in young women, and presentation is with abdominopelvic symptoms. The ovarian involvement is part of more widespread metastasis in the vast majority of cases.

Pathology

About half of all cases are bilateral and the average size is 10 cm. Only a third of cases are pigmented macroscopically, showing a black or brown color, sometimes only focally. Most tumors are predominantly solid although a significant proportion is at least focally cystic and sometimes extensively so; this may produce an appearance similar to "chocolate cysts" (Figs. 16.29 and 16.30).

Histologically about half of all cases are amelanotic. The tumors are usually composed of round cells; although these are arranged in solid sheets, the formation of follicle-like spaces is common and may impart an appearance similar to ovarian small cell carcinoma or other primary tumors. The tumors may consist of abundant eosinophilic cytoplasm and thereby resemble ovarian tumors with oxyphilic cells. Some tumors are composed of spindle cells and these may have a fascicular arrangement. Other tumors are composed of sheets of epithelioid cells, small cells, clear cells, or rhabdoid cells.

Differential Diagnosis

Distinction from a variety of ovarian primary neoplasms can be difficult in the absence of a known history. These tumors are well-known mimics of almost any tumor subtype known and in the ovary can resemble sex cord–stromal tumors of adult granulosa cell or steroid cell types or epithelial tumors of high-grade serous,

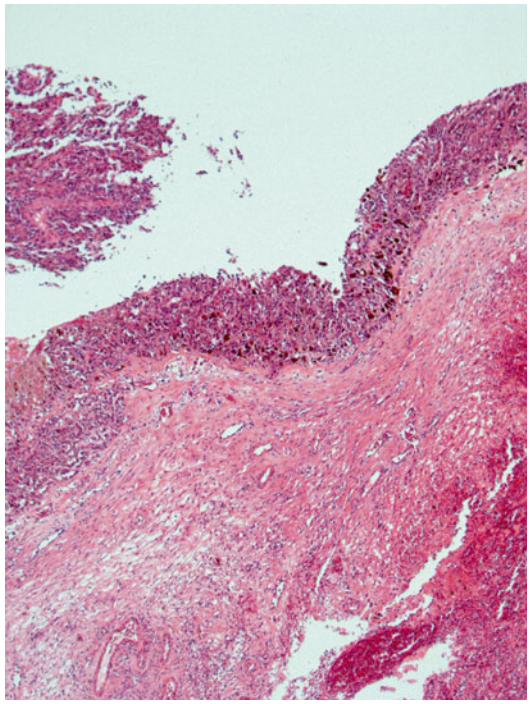

Fig. 16.29 Cystic metastases from malignant melanoma can produce a low-power appearance resembling endometriosis

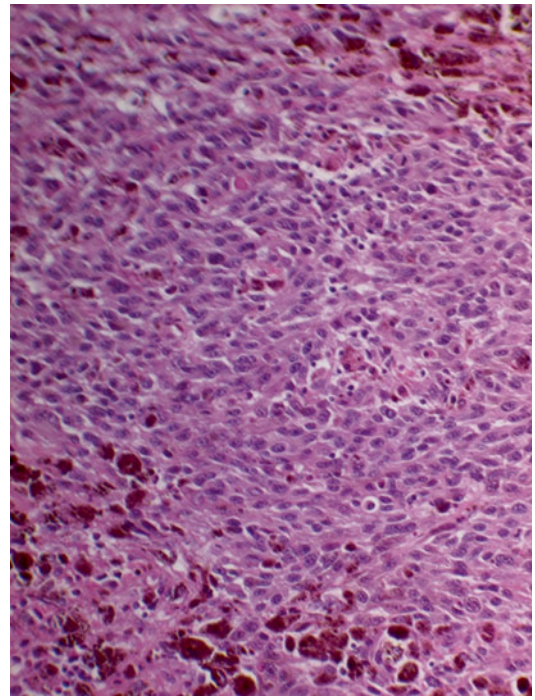

Fig. 16.30 Malignant melanoma metastatic to the ovary (Courtesy of Dr Nafisa Wilkinson, St James's Hospital, Leeds, UK)

clear cell, and undifferentiated types with a solid pattern. Other round cell tumors and spindle cell tumors may also fall in the differential diagnosis. If suspected, the diagnosis can usually be established using a panel of immunohistochemical markers including S100, HMB45, melan A, and others, together with demonstration of negative reactivity for markers of epithelial and sex cord–stromal differentiation, although focal positivity for inhibin and calretinin has been reported in melanoma. It is important for the pathologist to always consider a metastatic melanoma as a possibility in ovarian tumors which do not show features classical of a primary ovarian neoplasm.

One further issue is the distinction of metastatic melanoma from a primary ovarian melanoma which is very rare and usually encountered in association with a mature teratoma [59].

Metastatic Carcinoid Tumors

Clinical Features

Metastatic carcinoid tumor is rare in the ovary [60]. Most cases occur in women over 40 years, and the clinical features of a carcinoid tumor are present in 40 % cases. There is evidence of extraovarian disease in 90 % cases. The prognosis is poor and 75 % of cases are fatal within 5 years, in comparison with the almost uniformly benign behavior of primary ovarian carcinoids. Most arise from ileal primaries, but jejunal, cecal, pancreatic, and pulmonary origin are also recorded.

Pathology

The tumors are usually bilateral, solid, multinodular masses with a minor cystic component. Some tumors may show a yellow cut surface. Microscopically the tumors are most commonly insular and composed of large islands of neoplastic cells with a characteristic granular chromatin pattern. Acinar formation is seen at the periphery of the tumor islands, and the luminal spaces may show hyaline material or dystrophic calcification. Follicle-like spaces are often seen and may resemble primary tumors. The stroma may show hyaline fibrosis. A minority of cases show trabecular or solid patterns.

Differential Diagnosis

Metastatic carcinoids may mimic primary sex cord–stromal tumors, including Sertoli–Leydig and adult granulosa cell tumors. The presence of cell groups with acinar and follicle-like structures in a fibromatous stroma causes diagnostic confusion with a benign Brenner tumor or adenofibroma. Primary carcinoid tumors may occur on a background of a mature teratoma which may not be evident at first but should be carefully sought and excluded. Immunohistochemistry for neuroendocrine markers is useful to confirm the neuroendocrine differentiation but is of no value in establishing primary origin. It should be noted that CD56 immunoreactivity in isolation is of no use in this distinction, as this marker shows reactivity with a variety of neoplastic tumors especially those of sex cord–stromal origin; instead a panel of markers including chromogranin and synaptophysin should be applied.

Metastases from the Respiratory Tract

Clinical Features

Metastasis from lung tumors occurs at an average age of 47 years, and most cases occur in the presence of a known history of lung cancer. Significantly though, many tumors are discovered at the same time as the ovarian mass, and in 16 % of cases, the ovarian tumor was discovered 2–26 months before the lung primary [61]. In 40 % of cases the lung and ovary are the only sites of disease.

Pathology

Only a third of cases present with bilateral ovarian tumors with an average size of about 10 cm. The tumors show a solid or solid and cystic appearance with areas of necrosis. Surface involvement is uncommon. Histologically the most common subtype is small cell carcinoma, followed by adenocarcinoma and large cell carcinoma. Squamous cell carcinomas constituted a minority of all cases and are unlikely to metastasize to the ovary.

Differential Diagnosis

The morphology of these subtypes resembles that of the primary. Vascular invasion is usually prominent. Metastatic small cell carcinoma may also show follicle-like spaces which are seen with other solid tumors metastasizing to the ovary. There is overlap with many primary tumor types, and correct diagnosis depends on suspecting metastasis and confirming with specific immunohistochemical markers, including TTF-1 and napsin, as well as clinical correlation.

Metastases from the Kidney and Urinary Tract

Clinical Features

Renal clear cell carcinoma can rarely give rise to ovarian metastasis. This usually follows within 2 years of diagnosis of the primary, but can occur before its clinical manifestation, or many years after its diagnosis. Transitional cell carcinomas of the urinary tract may rarely metastasize to the ovary [3].

Pathology

Metastatic renal cell carcinoma is often unilateral and large, with a solid–cystic, yellow cut surface. The histology is very similar to primary ovarian clear cell carcinoma. Metastatic transitional cell carcinoma may be indistinguishable from a primary surface epithelial tumor.

Differential Diagnosis

In the absence of a known history of renal clear cell carcinoma, metastases can be difficult to distinguish from primary ovarian clear cell carcinomas. The primary tumors however often occur in a background of endometriosis, show a greater variation in cell morphology and architecture, and show prominent stromal hyalinization. By contrast metastatic renal clear cell carcinomas are more uniform and monotonous in their appearance. In both these tumors, i.e., renal cell carcinoma and transitional cell carcinoma, where a metastasis is being considered, a complete clinical history is essential to confirm the diagnosis.

Metastases from the Liver

Clinical Features

Excluding tumors of the intrahepatic bile ducts which have been considered above with pancreaticobiliary tumors, tumors of hepatic origin

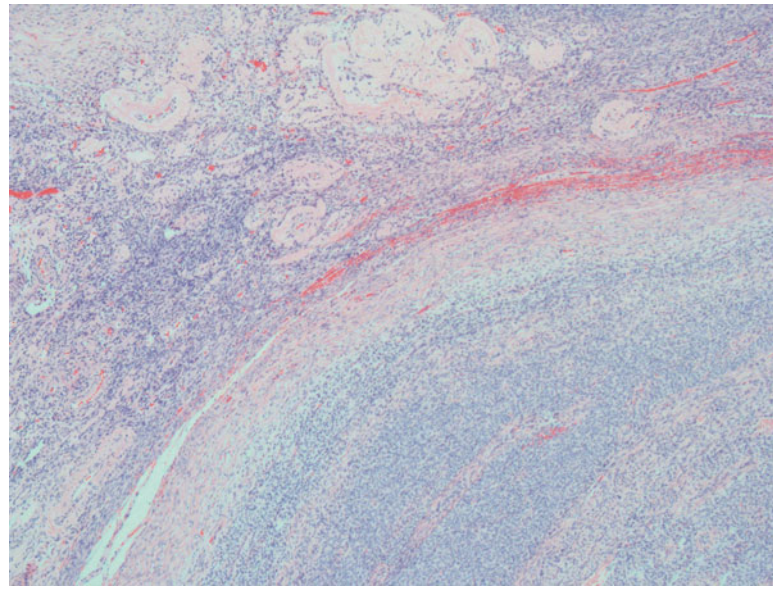

Fig. 16.31 Metastasis from low-grade endometrial stromal sarcoma many years after removal of primary

uncommonly give rise to ovarian metastases. These may occur in young individuals and present as an ovarian mass.

Pathology

These tumors may be bilateral and solid. Histologically hepatocellular carcinomas are composed of cells with abundant eosinophilic cytoplasm. Useful diagnostic features are the presence of bile in canaliculi.

Differential Diagnosis

These tumors can mimic a variety of oxyphilic tumors of the ovary including clear cell carcinoma, hepatoid carcinoma, or hepatoid yolk sac tumor. The primary tumor may be difficult to diagnose [3].

Metastatic Endometrial Stromal Sarcoma

Clinical Features

These are the most common sarcomas to metastasize to the ovary [3, 62]. This occurs in perimenopausal or postmenopausal women. A previous history of endometrial stromal sarcoma or other uterine neoplasm may be present, but as these tumors are indolent, this may be remote. They also need to be distinguished from a primary endometrial stromal sarcoma which may develop from endometriosis within an ovary.

Pathology

These tumors are bilateral solid masses with a soft yellow cut surface and at times a minor cystic component. It may be possible to appreciate the worm-like intravascular growth on naked eye examination. On microscopic examination, there is a diffuse pattern of oval cells with scanty cytoplasm (Figs. 16.31 and 16.32). Arterioles may not be conspicuous and the characteristic intravascular pattern may only be seen at the hilum if at all. There may be a prominent epithelial component and stromal hyalinization with hyaline plaque formation.

Differential Diagnosis

There is morphological overlap with sex cord–stromal tumors, principally diffuse adult-type granulosa cell tumors and fibrothecomas. Positive immunohistochemistry for CD10 is also seen in sex cord–stromal tumors, and so this marker should be accompanied by a panel of markers more specific for sex cord differentiation to avoid misdiagnosis. Endometrial stromal sarcoma can also occur as an ovarian primary often in a background of endometriosis.

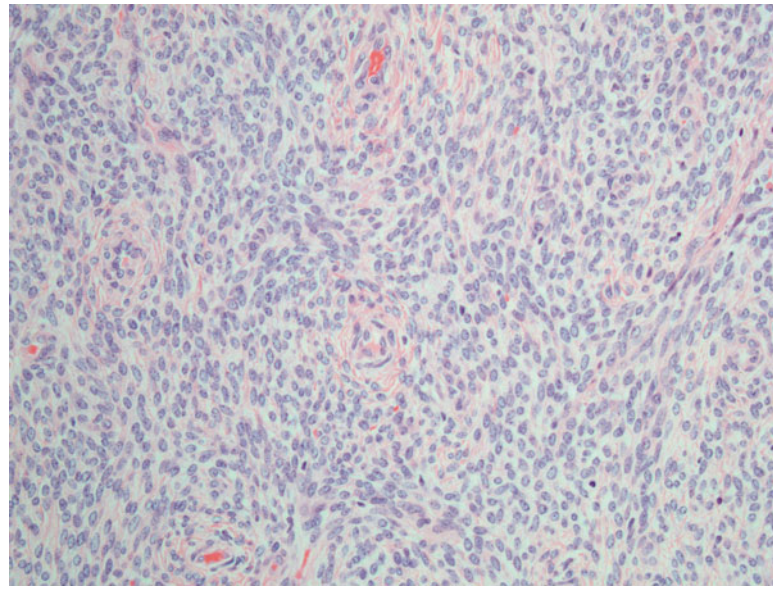

Fig. 16.32 Metastatic endometrial stromal sarcoma composed of monotonous bland elongated cells with prominent arteriolar vessels

Metastatic Gastrointestinal Stromal Tumor

Clinical Features

Gastrointestinal stromal tumors are being increasingly recognized as a diagnostic problem for gynecological pathologists. These may masquerade as primary smooth muscle tumors when they occur in the rectovaginal septum or present with ovarian metastases simulating an ovarian primary [63, 64]. These occur in middle aged to elderly women and may be discovered before or many years after the primary which is usually in the small bowel or mesentery.

Pathology and Differential Diagnosis

These tumors are cellular spindle cell masses indistinguishable from primary smooth muscle tumors or cellular fibromas. Positive immunohistochemistry for c-kit or DOG1 clinches the diagnosis if this is suspected.

Metastases from Mesothelial Tumors

Clinical Features

Malignant mesothelioma of the peritoneum may show ovarian involvement in a significant number of cases, resembling widely metastatic ovarian serous carcinoma. The patients show a wide age range of 17–92 years, and presentation with abdominopelvic symptoms including ascites and a pelvic mass [65] may be encountered. Intra-abdominal desmoplastic round cell tumor is a rare mesothelial tumor of uncertain histogenesis that can also present with ovarian involvement in young women [66].

Pathology

The majority shows an epithelial morphology with only rare cases being biphasic or sarcomatoid; a few show deciduoid features [65]. The tumor has a tubulopapillary architecture and shows prominent stromal hyalinization. The epithelial cells show monotonous nuclei with mild atypia.

Desmoplastic round cell tumor involving the ovary is composed of round cells separated by stroma in an insular pattern.

Differential Diagnosis

These tumors closely resemble primary serous tumors although the latter show more cellular stromal cores in papillary areas and greater cytological variation than the monotonous appearance in mesotheliomas. Psammomatous calcification is not as prominent in mesothelioma while this is a more common feature in serous tumors of all types. Immunohistochemistry for the mesothelial markers calretinin and D2-40 is useful [3, 65].

Desmoplastic round cell tumor resembles small cell carcinoma, sex cord–stromal tumors, and metastatic lobular carcinoma. Immunohistochemistry is essential for correct diagnosis of ovarian round cell tumors [66, 67].

Pathology of Synchronous Primary Endometrial and Ovarian Carcinomas

Introduction

About 1–2 % of all women with gynecological cancers have two or more simultaneous independent primary tumors involving the female genital tract [68–70]. The female genital tract develops from the Mullerian ducts, invaginations of the coelomic cavity. There are a number of genes involved in the differential development of various parts of the tract whose expression in turn is regulated by a variety of local and systemic influences, principally ovarian hormones as well as inflammatory and immune modulators [71]. The sensitivity of the female genital tract to such humoral influences is manifest during its development and also throughout reproductive life as cyclical and pregnancy-related changes occur. The peritoneal mesothelium appears to retain its capacity to differentiate along Mullerian lines. Endometriosis and endosalpingiosis are likely results of such aberrant or ectopic differentiation, although other theories exist for their development. Such foci, under the influence of relevant tumorigenic stimuli, could develop tumors as a result of field change. It is currently believed that many gynecological malignancies arise from the secondary Mullerian system [72].

Synchronous tumors of the endometrium and ovary account for 50–70 % of all synchronous female genital tract malignancies [68, 70]. About 10 % of women with ovarian cancer will be found to have synchronous endometrial cancer, and about 5 % of women with endometrial cancer harbor simultaneous ovarian cancer [73]. A higher incidence of synchronous tumors is reported in patients of endometrial cancer aged under 50 years [74, 75].

Clinical Features

The median age reported in various series ranges from 41 to 52 years, about a decade younger than the median age of incidence of either endometrial or ovarian cancer alone [73, 76–82]. The median age is younger for tumors with endometrioid histology than other types [73]. The median BMI is reported to be high in the largest single series from one institution [73] with a range of 15.5–53 [73, 79], and over a third of women are obese. About two-thirds of women with synchronous endometrioid tumors are premenopausal [73, 79] and roughly 40 % are nulliparous [73, 83]. The most common presentation is abnormal uterine bleeding with the ovarian carcinoma being discovered secondarily [68, 73, 76, 79, 84]. A minority of cases present with a pelvic mass, pelvic pain, or other symptoms.

Pathology

The endometrial and ovarian tumors reported in most large series are both of endometrioid type in roughly 50–70 % of cases. The remainder show mixed histology or different histology at the two sites. Owing to the common occurrence of mixed tumors at both sites, these need careful evaluation as tumors of apparently different histologies may still represent metastases with a minor component being missed at the primary site.

As a group these tumors tend to show grade 1 or 2 endometrioid carcinoma in both endometrium and ovary with approximately 70 % concordance in grade [85]. The endometrial carcinomas tend to be grade 1 or 2 in nearly 90 % cases and are generally confined to the endometrium or show superficial myoinvasion (Fig. 16.33). Deep myoinvasion is seen in a minority of cases [73]. Vascular invasion is present in about 30 % of cases and is considered a poor prognostic sign [78]. Ovarian tumors range from <4 cm to over 10 cm in diameter; roughly one quarter of the ovarian tumors are discovered in normal-sized ovaries. These are generally solitary with no surface involvement [73] (Fig. 16.34). Associated ovarian endometriosis is seen in about 30 % of cases [85].

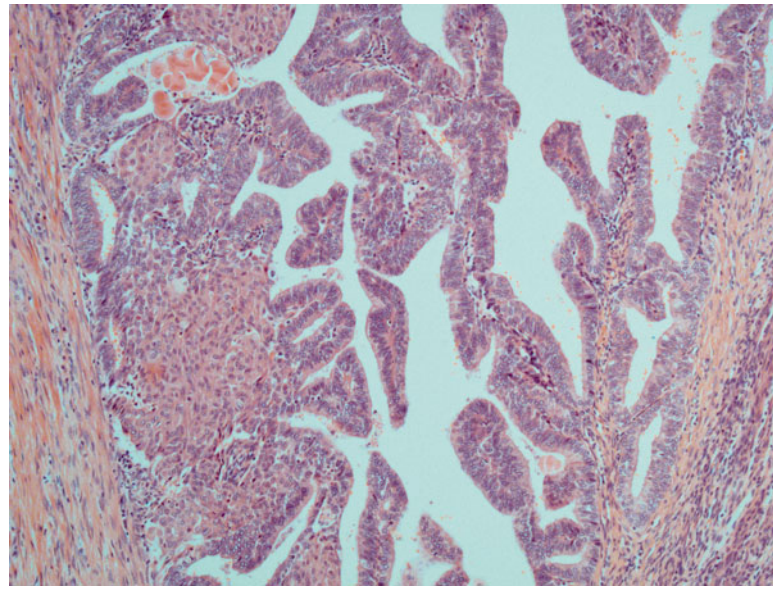

Fig. 16.33 Grade 1 endometrioid endometrial carcinoma with synchronous carcinoma in the ovary, seen in Fig. 16.34

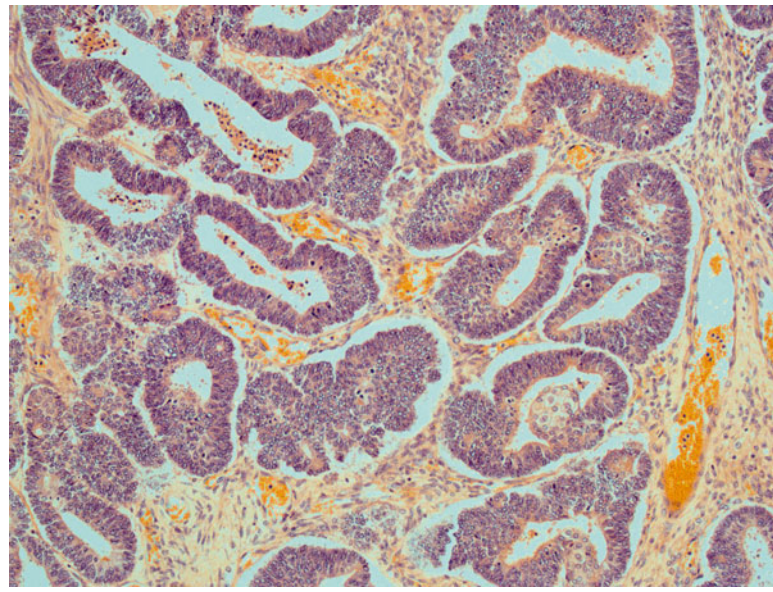

Fig. 16.34 Synchronous grade 1 endometrioid ovarian carcinoma in the case illustrated in Fig. 16.33

Differential Diagnosis

The main diagnostic consideration is exclusion of metastasis from an endometrial primary to the ovary or vice versa. Criteria for distinguishing synchronous tumors from metastases were first documented by Ulbright and Roth [86] and subsequently detailed by Scully et al. [13]. These are widely accepted and summarized in Table 16.3. Although criteria have also been laid down for ovarian carcinoma metastasizing to the endometrium [13], metastasis from the ovary preferentially to the endometrium in the absence of other pelvic involvement is an exceedingly unlikely occurrence with very exceptional cases documented in the literature [86]. Such spread would generally include involvement of the corpus from the external surface, and tumors with these features have been excluded from most series as they do not pose a diagnostic problem.

Table 16.3 Comparison of features of synchronous independent endometrial and ovarian carcinomas with those of endometrial carcinomas with ovarian metastasis

Feature	Synchronous independent endometrial and ovarian carcinomas	Endometrial carcinomas with ovarian metastasis
Histological type	Usually both endometrioid, may be entirely different histological types	Similar and consistent with endometrial primary
Histological grade	Both low grade	Similar and consistent with endometrial primary
Myometrial invasion	None or minimal	Usually deep myometrial invasion
Tumor in cervix	Absent	Often present
Tumor in Fallopian tubes	Absent	Often present
Vascular invasion	Absent	Often present
Uni-/bilateral ovarian tumors	Unilateral	Often bilateral
Ovarian endometriosis	Present	Usually absent
Pattern of ovarian involvement	Single dominant mass	Multinodular with surface involvement
Local extension	Both tumors confined with no direct extension to contiguous sites	May show large endometrial tumor with direct extension to ovaries
Involvement of other sites	Both tumors confined with no spread to sites beyond the endometrium and ovary	Involvement of other sites may be present
Molecular changes in tumors at the two sites	Typically dissimilar	Similar or identical

Molecular Changes in Synchronous Endometrial and Ovarian Tumors

It has been shown that the majority of tumors can be accurately categorized by histological evaluation as synchronous primaries or single primary with metastasis, as demonstrated by the poorer clinical outcome of the latter. Unfortunately there remain a few cases that cannot be classified with confidence, either because of widespread involvement, in which case the distinction is of academic rather than practical or prognostic significance, or, because of, more importantly, overlapping or ambiguous histological features.

Molecular analysis of synchronous endometrial and ovarian tumors has been the subject of a large number of research publications. The vast majority are aimed at finding robust molecular diagnostic tests that can complement histopathology or provide an alternative or more accurate diagnosis in difficult cases. A second approach is for studying the molecular pathogenesis of this enigmatic group of tumors. A few studies are specifically devoted to the association between microsatellite instability in the context of the association of HNPCC with the development of synchronous tumors; these are discussed in a later section.

The techniques that have been assessed for diagnosis of synchronous tumors as independent primaries range from ploidy analysis by Feulgen [87] or flow cytometry [88, 89], X chromosome inactivation studies [90], loss of heterozygosity (LOH) [91–93], microsatellite instability (MSI), mitochondrial DNA genotyping [94], beta-catenin expression, and gene-specific analysis of p53, k-ras, pTEN, and beta-catenin mutations [78, 90, 95–101]. Recent studies promote detection of multiple genetic changes in tumors to minimize the confounding effects of tumor progression and heterogeneity. The most reliable markers are generally those that are present early in tumor development. Major advances in molecular biological techniques allow detection of multiple genetic changes using gene expression and/or DNA microarrays or microsatellite analysis. In a study of 90 cases of simultaneous endometrial and ovarian cancers, it was found that histology alone provided a diagnosis in only 61 % of cases while the combination of histology and molecular diagnosis based on LOH at 22 loci and MSI was able to categorize 98 % of cases

[93]. There was 91 % concordance between histology and molecular results. In a similar study on 12 cases using these techniques together with pTEN and CTNNB1 mutations and beta-catenin expression, it was found that 5 of 8 cases diagnosed as independent primaries showed at least one different molecular alteration [101]. It was noted, however, that there was also at least one or more identical alteration in these pairs of tumors. These results are similar to those of previous studies [78, 97, 98]. An additional observation of potential routine diagnostic value is the frequent presence of CTNNB1 mutations in cases of independent primary endometrial (50 %) and/or ovarian (44 %) tumors. This is associated with nuclear as opposed to membranous expression of beta-catenin on immunohistochemical analysis. The staining pattern of beta-catenin may therefore be a valuable additional diagnostic tool in differential diagnosis as it is more likely to be associated with synchronous independent primary tumors than those occurring singly and is associated with better outcome [97, 101]. This finding, however, needs wider testing.

A second approach to identify the organ of origin has been the use of gene expression profiling. The identification of single genetic alterations is unreliable for diagnosis because of a large degree of overlap in signature genetic abnormalities in endometrial and ovarian primary endometrioid carcinomas. In a recent study gene expression profiling demonstrated 163 genes that showed differential expression in endometrioid cancers of endometrial and ovarian origin, enabling generation of a 119-gene predictive model. This showed concordance with histopathology in 11/16 cases. Further studies are needed to determine the practical utility of this technology in a prospective setting [102].

Some molecular studies have also tried to elucidate the molecular pathogenesis of synchronous tumors. These have aimed to evaluate whether this is a coincidental occurrence or whether such tumors have a different pathogenesis from sporadic cases. The most frequent molecular abnormalities underlying endometrioid carcinoma of the uterus are mutations in pTEN (30–50 %) and the beta-catenin gene (25 %), as well as microsatellite instability (MI) (20–45 %), which is due to hMLH1 promoter gene hypermethylation in sporadic cases as opposed to the specific mismatch repair gene mutations occurring in HNPCC carriers. The same abnormalities are reported in ovarian endometrioid tumors, though with different frequencies [103], and hence are not of diagnostic value. In synchronous endometrioid tumors of the endometrium and ovary, MI is reported to occur at about double the frequency seen in single tumors, together with a higher rate of pTEN and CTNNB1 mutations. It is suggested that these three mutator pathways are all of importance in the development of synchronous tumors and that the pathogenesis of these tumors is different from that of single primaries [97, 101].

Results of molecular studies should be cautiously interpreted and always together with clinicopathological findings. The finding of different genetic abnormalities may reflect tumor heterogeneity rather than evidence of separate primaries [104]. On the other hand, identical gene mutations have been detected in clearly independent primaries, probably resulting from a "field effect" of a common oncogenic stimulus [101]. Similarly loss of heterozygosity, though generally considered an early reflection of loss of tumor suppressor genes, may also appear as a late event due to genetic instability [96, 105].

Prognostic Factors

Several studies have compared outcome of synchronous primary tumors with that of endometrial cancer with ovarian metastasis as categorized by histological findings. These have consistently demonstrated vastly superior survival in cases of synchronous endometrioid primaries. This has given conclusive evidence that these tumors are indeed low-stage independent primaries, and it is advocated that such tumors, if confined to the endometrium and ovary, do not require adjuvant treatment following surgery. A large prospective GOG study that did not distinguish between synchronous and metastatic tumors but was designed to study clinical outcome found that the groups as a whole showed better survival than either

stage 3 endometrial or stage 2 ovarian cancers [85]. Within the group factors influencing survival were found to be the presence of metastasis outside the ovaries and tumor grade. Poor prognostic factors highlighted in other studies are the presence of deep myoinvasion [106], vascular invasion [107], stage of the ovarian cancer [108], positive washings, and tumor grade [109, 110].

Many studies have compared the outcome in synchronous tumors of endometrioid type with that of other subtypes. Although some showed no significant differences in clinical features or survival [79, 106, 111, 112], the majority of large studies have demonstrated significantly better outcomes in cases of synchronous endometrioid tumors as opposed to other subtypes, and many studies have excluded uterine serous and clear cell carcinoma and carcinosarcoma from their series. These "type 2" cancers are generally seen in older postmenopausal women and tend to behave aggressively. Uterine serous carcinoma even when presenting with "apparently" low-stage disease within the uterine corpus may have widely disseminated peritoneal disease. For these reasons it is unlikely that "type 2" tumors simultaneously involving the endometrium and ovaries ever represent independent primaries. Molecular studies are also confounding in these cases as there is a high degree of tumor heterogeneity and rapid progression in genetic abnormalities. Since such subtypes are treated aggressively irrespective of stage, it is unlikely that histological misinterpretation will influence management adversely.

Synchronous Endometrial and Ovarian Cancer in Young Women

It has been noted that the median age for synchronous independent endometrial and ovarian cancers is about a decade lower than that of single primaries at either site. While synchronous tumors of the ovary are reported in 5 % of endometrial cancers overall, this incidence is significantly higher in women under 50. Two studies have reported that the incidence of a synchronous ovarian tumor is 19 % in women under 50 [74] and 25 % in women aged 24–45 [75]. In 15 % of cases the involved ovaries were normal or benign appearing on radiological and/or intraoperative assessment [75]. Women with endometrial cancer who desire ovarian conservation need to be appropriately counseled, and the high risk of synchronous ovarian tumors should be discussed.

Synchronous Endometrial and Ovarian Cancer and HNPCC

The syndrome of hereditary non-polyposis colorectal cancer (HNPCC) is caused by mutations in a family of genes known as DNA mismatch repair (MMR) genes, most commonly hMLH1 or hMSH2. This results in progressive accumulation of DNA replication errors in the progeny of an abnormal cell and genetic instability; the molecular evidence of this phenomenon is the demonstration of widespread microsatellite instability. Individuals with HNPCC are at increased risk of development of a variety of cancers: endometrial, ovarian, stomach, small intestine, hepatobiliary tract, ureter, brain, and skin. A number of studies have shown that women with HNPCC are at a higher risk of developing endometrial cancer than colorectal cancer; in comparison with a lifetime risk of around 50 % for colorectal cancer, women with HNPCC have a lifetime risk of 40–60 % for endometrial cancer and 10–12 % for ovarian cancer [113]. Current guidelines recommend that patients with two HNPCC-associated cancers should undergo appropriate screening [114].

Overall MMR gene mutations account for a tiny minority of endometrial cancers [113]. Two studies devoted to identifying the prevalence of HNPCC in synchronous endometrial and ovarian cancers found these to account for 3 and 7 % of cases, even after limiting analysis to younger patients [114, 115]. This was comparable to that in endometrial cancers in general and considerably lower than that in cases of synchronous or metachronous endometrial and colorectal cancer. Both studies concluded that since this is a relatively common tumor combination to occur sporadically, genetic testing in

synchronous endometrial and ovarian cancers should be limited to cases with a suggestive family history. For the diagnostic pathologist, it is sufficient to raise this as a possibility in a multidisciplinary setting, and immunohistochemical testing for MMR gene product expression may not be indicated in all cases as the yield is likely to be low.

Summary and Practical Approach to Synchronous Endometrial and Ovarian Cancers [116]

A synchronous tumor in the ovary or endometrium is seen, respectively, in 5 % of women with endometrial cancer and 10 % of women with ovarian cancer. The distinction of synchronous endometrial and ovarian primaries from stage 3 endometrial cancer is crucial for correct patient management. The diagnosis of synchronous primaries should be made with extreme caution, if at all, in grade 3 endometrioid and type 2 endometrial cancers. For endometrioid cancers, most cases can be accurately categorized on the basis of standard histological features summarized in Table 16.3.

Molecular testing can provide valuable adjunctive information but must be interpreted with clinicopathological correlation and not in isolation. Gene expression profiling may provide specific diagnostic information for accurate staging of synchronous tumors in the future. Nuclear, as opposed to membranous, expression of beta-catenin on immunohistochemistry is reported to favor synchronous independent primaries but requires wider testing for confirmation of this observation. Poor prognostic features are tumor grade, vascular invasion, and stage-related factors: deep myoinvasion, positive peritoneal washings, and metastasis outside the uterus and ovaries. A very low percentage of women with synchronous primaries in the uterus and ovary are HNPCC patients, and genetic or immunohistochemical testing for mismatch repair gene mutations may be unnecessary in all cases, but should be carried out according to prevailing guidelines. Women under 50 are much more likely than older patients to have a synchronous ovarian tumor, and this should be taken into account if ovarian conservation is being considered.

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Pathology of the Fallopian Tube

17

Philip P.C. Ip and Annie N.Y. Cheung

Abstract

The fallopian tubes are subjected to a variety of inflammatory and neoplastic lesions. Reactive changes secondary to inflammation may potentially mimic malignancy. Many reactive and benign conditions may also result in tubal narrowing or occlusion and are often the underlying causes of ectopic pregnancy. The tube is the most frequent site of an ectopic pregnancy but is rare for primary gestational trophoblastic diseases. The latter are usually secondary to a lesion from the corpus. Tubal hyperplasia is a poorly defined and overlapping entity; in many cases, they represent precursor lesions of tubal borderline or malignant lesions. Recent advances in genetic testing for BRCA mutations in high-risk families had led to the recognition of early carcinomas of the fallopian tube in prophylactic bilateral salpingo-oophorectomy specimens. Tubal intraepithelial carcinoma has become the topic of vigorous research in recent years, and this lesion, particularly when found in the fimbriae, is now recognized as the source some ovarian and peritoneal high-grade serous carcinomas. Secondary involvement of the tubes by ovarian carcinomas or those of other sites is more common than primary tubal carcinomas. The commonest primary carcinomas of the fallopian tube are of serous differentiation, followed by endometrioid and transitional cell. Mesenchymal tumors are rare. Benign and malignant tumors of the broad ligament are similar to those arising from the ovaries in many aspects. Identification of sites of origin may sometimes be difficult.

Infections and Inflammatory Lesions

Acute Salpingitis

The presence of acute inflammatory cells may be seen in the fallopian tube under physiological conditions such as menstruation and puerperium due to a reaction to the reflux of uterine content.

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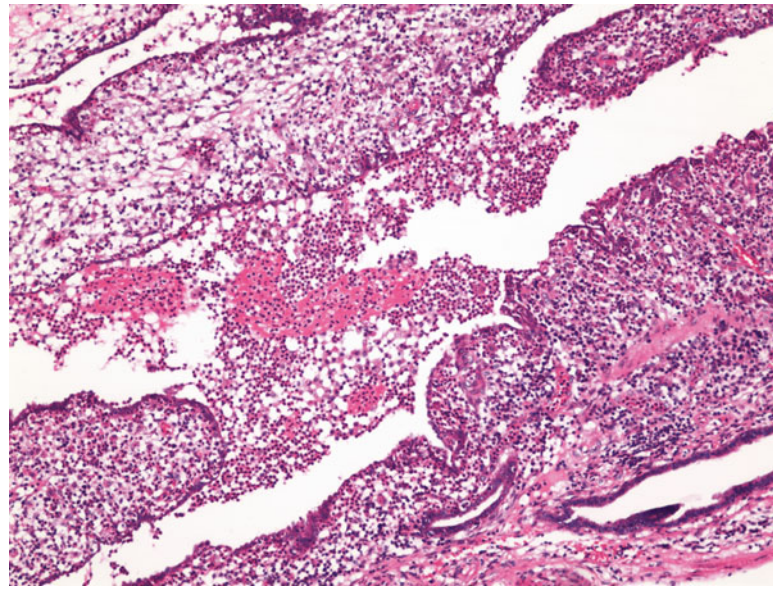

Fig. 17.1 Acute salpingitis. The tubal wall is diffusely infiltrated by acute inflammatory cells. The tubal plicae are edematous and congested

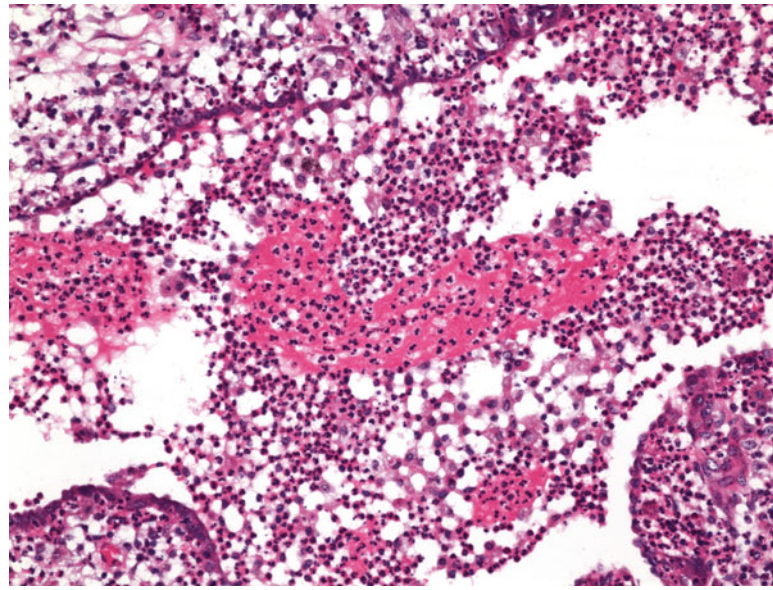

Fig. 17.2 Acute salpingitis. The tubal lumen is filled with neutrophils and fibrin

Under these circumstances, the inflammatory infiltrate is usually confined to the mucosa, and the myosalpinx is otherwise unremarkable. In contrast to salpingitis secondary to an infection, the tube may be dilated and congested, and the serosa is usually covered by inflammatory exudate. In severe cases, there may be a tubo-ovarian abscess. Microscopically, the neutrophilic infiltrate fills the tubal lumen and infiltrates the entire thickness of the wall (Figs. 17.1 and 17.2). The tubal plicae may fuse and may cause obstruction. The causes of acute salpingitis in many cases are sexually transmitted, such as gonorrhea, trichomoniasis, and chlamydial and mycoplasma infections [1]. It may also be secondary to abortions or complicated pregnancies in which the infections are usually caused by streptococci, staphylococci, or enteric bacteria. The intrauterine contraceptive device is an uncommon cause of acute salpingitis, and the causative organism is sometimes due to *Actinomyces israelii*, a Gram-positive

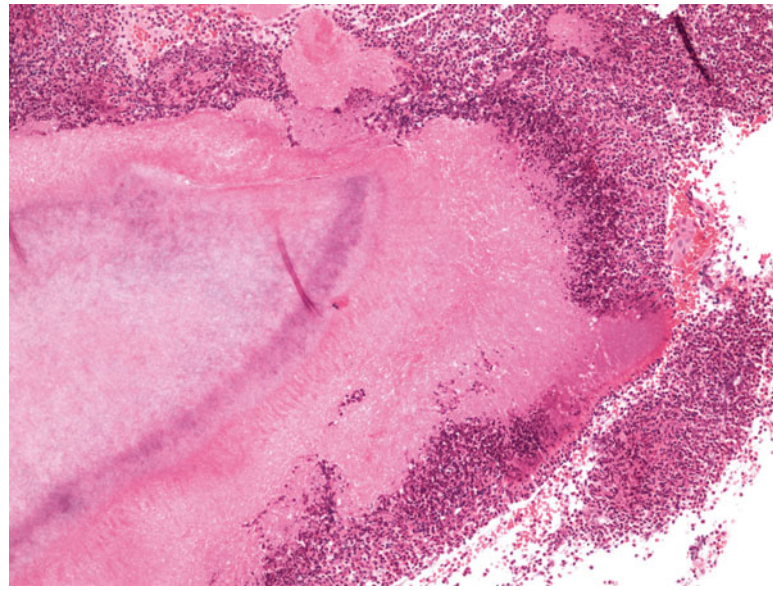

Fig. 17.3 Actinomycosis. A typical colony of *Actinomyces israelii*

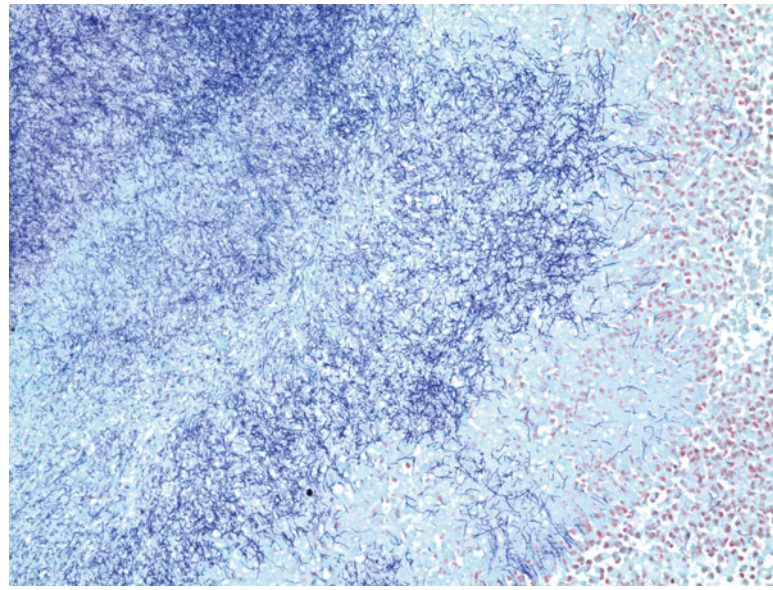

Fig. 17.4 Actinomycosis (Gram stain). *Actinomyces israelii* is a Gram-positive Actinobacteria

Actinobacteria (Figs. 17.3 and 17.4). In actinomycosis, the typical colonies of *Actinomyces* ("sulfur granules") are accompanied by marked suppurative inflammation.

Chronic Salpingitis

With the resolution of the acute inflammation and healing in acute salpingitis, the pus may undergo proteolysis to become a thin and serous fluid. There may also be significant fibrous adhesion, including fusion of the tubal plicae and other parts of the wall, and results in narrowing and occlusion of lumen, follicular salpingitis, and even hydrosalpinx (Fig. 17.5). The inflammatory cells are usually lymphocytes and plasma cells, but eventually these will disappear with resolution. A hydrosalpingiotic tube can potentially mimic an ovarian cyst.

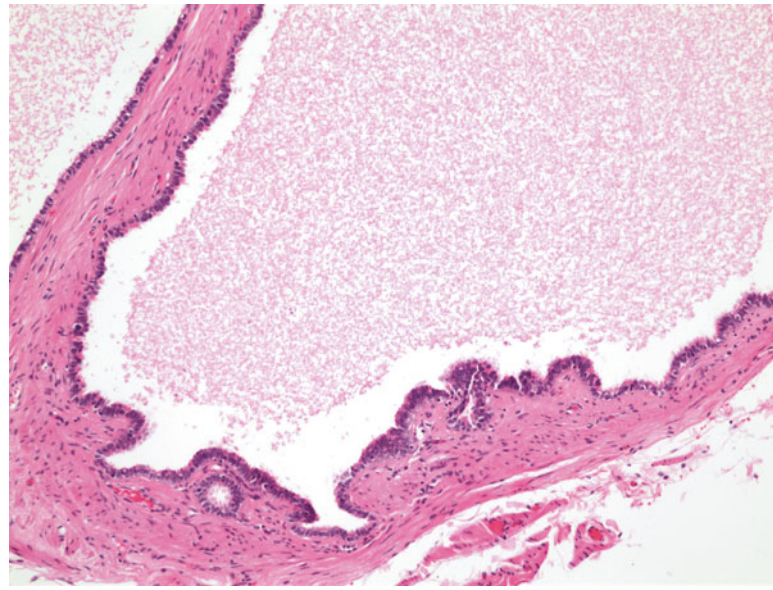

Fig. 17.5 Hydrosalpinx. This may be a sequel of chronic salpingitis. The lumen is distended with amorphous eosinophilic fluid, the tubal plicae are attenuated, and the myosalpinx is stretched and becomes thin

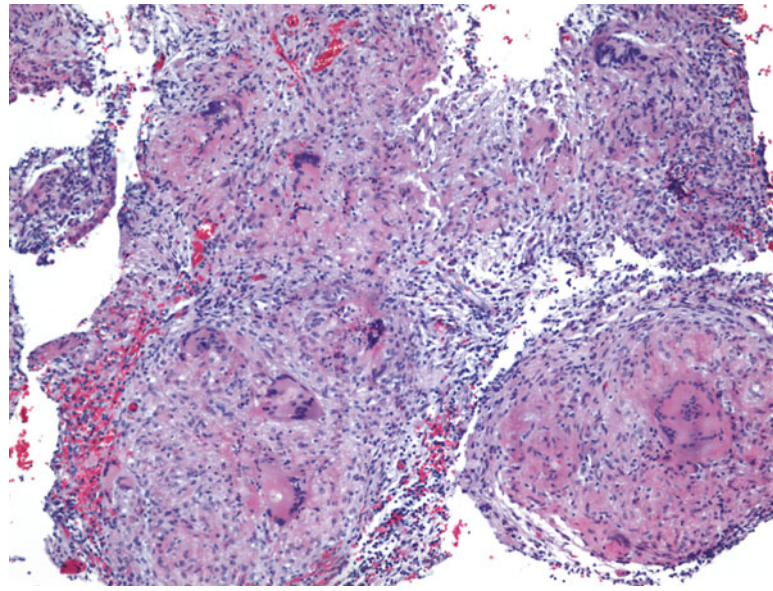

Fig. 17.6 Tuberculous salpingitis. The tubal plicae are ulcerated, with the cores replaced by granulomas

Tuberculous Salpingitis

Genital tuberculosis is usually secondary to pulmonary tuberculosis, which is spread by either a hematogenous or lymphatic route. It is a common cause of infertility in developing countries. The patients usually present with pain, menstrual disturbances, infertility, or combinations thereof. The gross appearance may resemble that of either acute or chronic salpingitis, but presence of caseous material is typical. Bilateral disease is common. Histologically, caseating granulomas with varying degrees of necrosis and fibrosis are seen (Figs. 17.6 and 17.7). Chronic cases usually lead to fusion of the plicae and obliteration of the tubal lumen, potentially leading to infertility or ectopic pregnancy. Although tuberculous infection is the most common cause of granulomatous salpingitis, some cases may be related to foreign bodies such as lubricants and starch granules. Though rare, fungal infections and parasitic infestations, Crohn's disease, and sarcoidosis may elicit a granulomatous reaction in the tube.

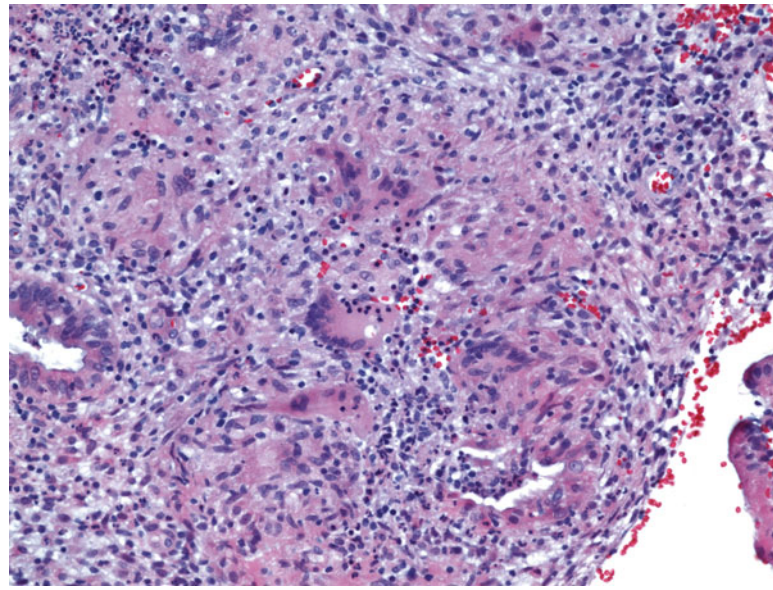

Fig. 17.7 Tuberculous salpingitis. The granulomas are composed of Langhans' giant cells and epithelioid histiocytes

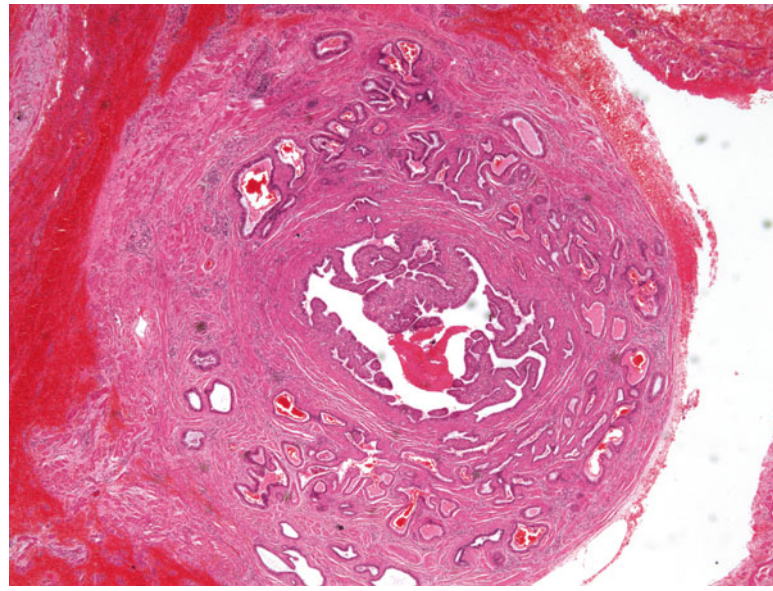

Fig. 17.8 Salpingitis isthmica nodosa. Cystically dilated glands are scattered throughout the whole thickness of the tubal wall, creating a pseudoinvasive appearance

Nonneoplastic Alterations

Salpingitis Isthmica Nodosa

This lesion is of uncertain pathogenesis. It usually involves the isthmus and is bilateral in 80 %. It affects women with a mean age of 30 years and these patients often have a history of infertility or present with ectopic pregnancy [2–4]. Grossly, there is often a yellow-white nodule measuring up to 2 cm but may be inconspicuous. Histologically, the lower magnification appearance resembles adenomyosis. Glands of various sizes and with irregular outline scattered throughout a hyperplastic and disorganized muscular wall (Figs. 17.8 and 17.9). These glands and cysts usually communicate with the tubal lumen, analogous to diverticula. They are lined by ciliated tubal epithelium. Distinction from carcinoma lies in the absence of cytologic atypia and stromal desmoplasia. It is also distinguished from endometriosis by the lack of associated endometrial stroma (Fig. 17.10).

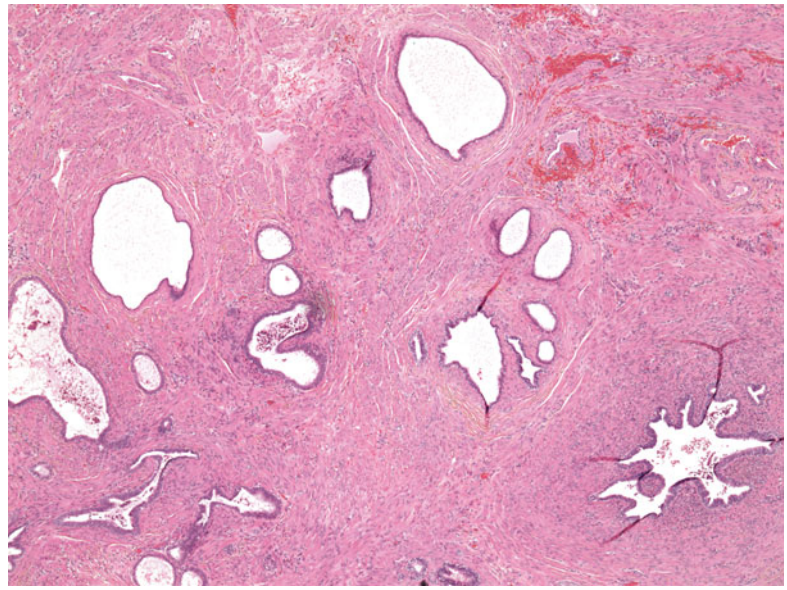

Fig. 17.9 Salpingitis isthmica nodosa. The glands are haphazardly distributed among the muscularis layer. There is no stromal desmoplasia

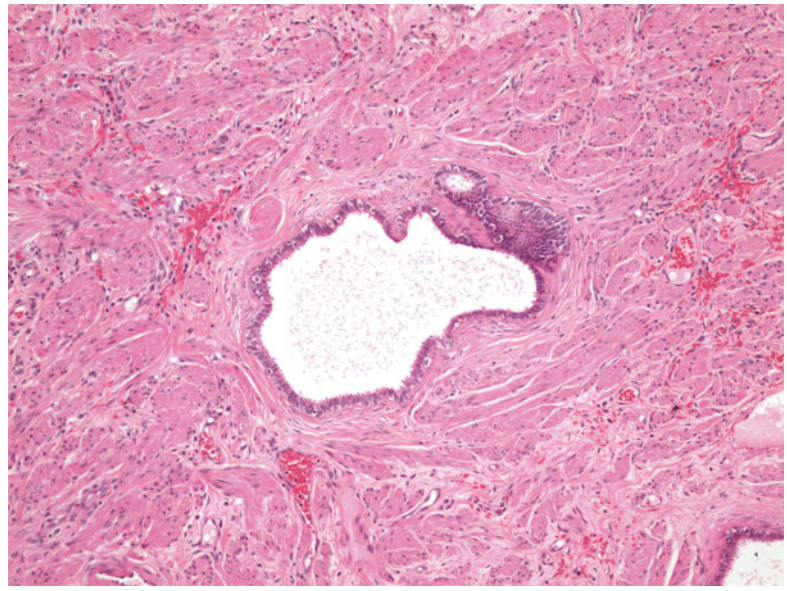

Fig. 17.10 Salpingitis isthmica nodosa. The glands are lined by a layer of tubal epithelium. Note the absence of periglandular cuff of endometrial stroma, distinguishing it from endometriosis

Endometriosis

The presence of endometrial-type glands and stroma in the tube is usually found in women of reproductive age who present with dysmenorrhea, pelvic pain, and infertility (Figs. 17.11, 17.12, 17.13, and 17.14). Endometrial lining may extend from the uterine cornu to the interstitial and isthmic portion of the tube, and this is considered physiologically normal by some. However, this may give rise to endometrial polyps [5–7]. Endometriosis involving the distal ends of the tubes may involve the mucosa or serosa. Those involving the mucosa only may be bilateral and account for 15–20 % of infertility and may lead to ectopic pregnancy [7]. Endometriosis primarily involving the tubal serosa is usually associated with

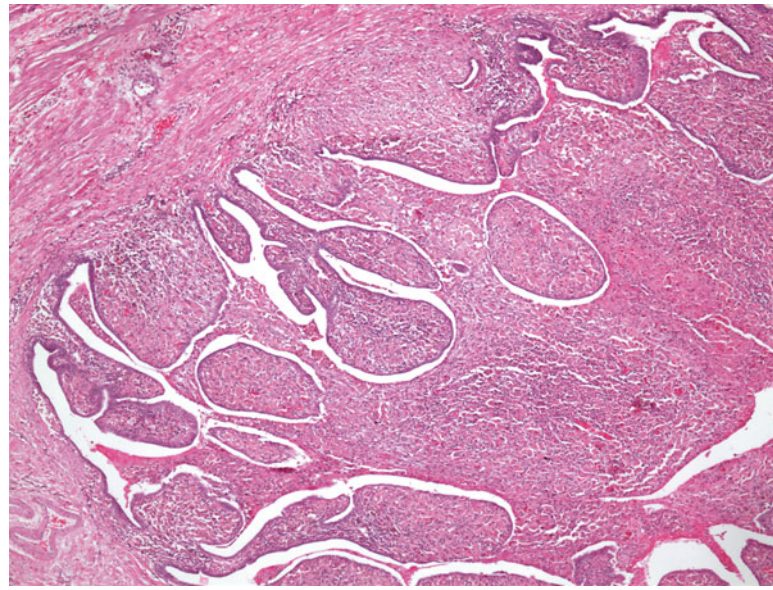

Fig. 17.11 Endometriosis. The tubal plicae are thickened and became club shaped. The tubal lumen is filled with altered blood and macrophages

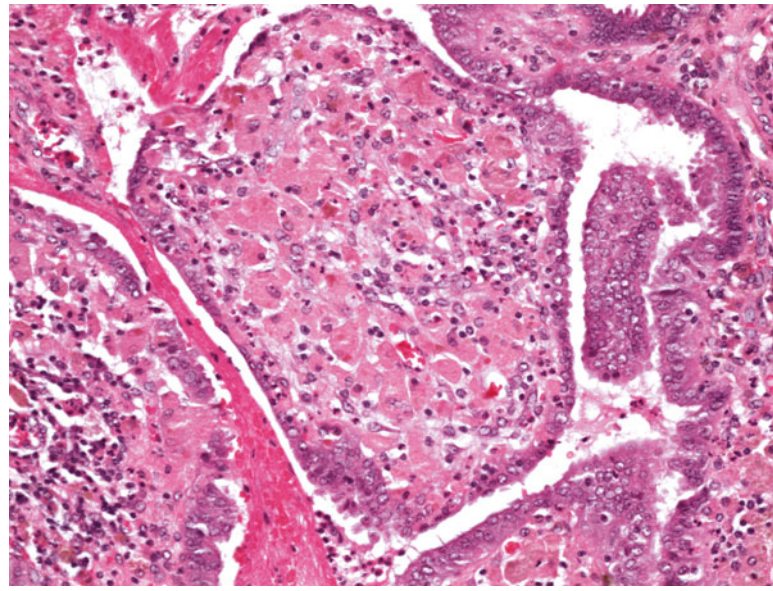

Fig. 17.12 Endometriosis. The cores of the tubal plicae are filled with hemosiderin-laden macrophages. Endometrial-type stroma is indiscernible

a more widespread pelvic endometriosis. Post-salpingectomy endometriosis occurs at the tip of the proximal tubal stump typically 1–4 years after tubal ligation. In these cases, the glands and endometrial-type stroma containing small vessels associated with recent hemorrhage are found involving the mucosa and the muscularis layer and, occasionally, the serosa. Rarely, this may lead to the development of tuboperitoneal fistulas [8, 9].

Tubal endometriosis may undergo decidual change or Arias-Stella change as a response to intrauterine or ectopic pregnancy. The epithelial cells of the endometriotic foci may occasionally contain abundant eosinophilic cytoplasm and large atypical nuclei [10]. This change is probably reactive in most cases and must be distinguished from tubal dysplasia or serous tubal intraepithelial carcinoma. Finding of any subtle endometrial-type stroma

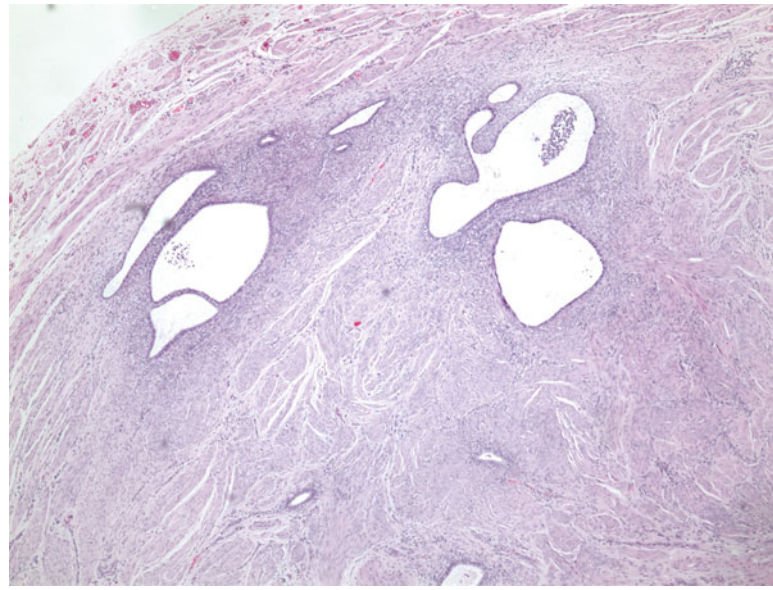

Fig. 17.13 Endometriosis. Foci of endometriosis involving the myosalpinx

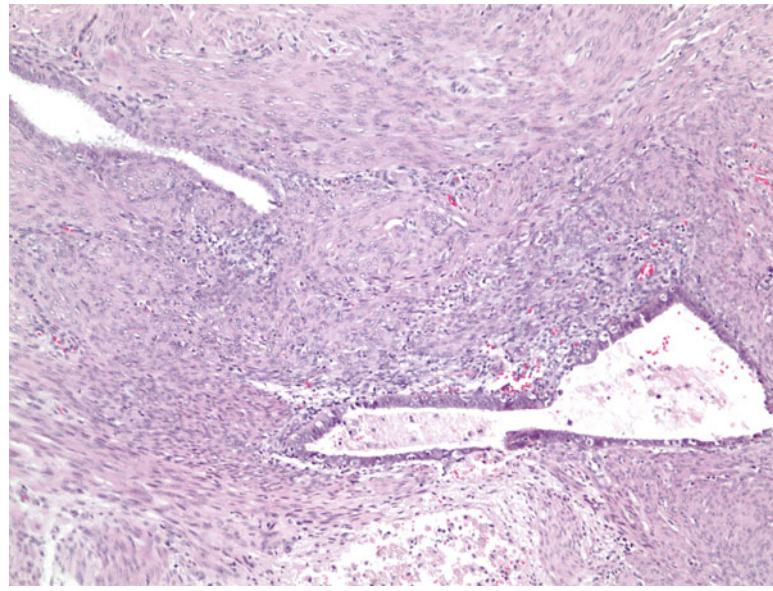

Fig. 17.14 Endometriosis. The glands are surrounded by endometrial-type stroma. In contrast to salpingitis isthmica nodosa (see Fig. 17.10)

would help confirming the diagnosis of endometriosis. CD10 immunostain may be helpful (Figs. 17.15 and 17.16) [11]. The stromal cells may undergo a peculiar "signet-ring cell decidual change." In this condition, the decidualized stromal cells may contain one or more cytoplasmic vacuoles, compressing onto the nucleus. In contrast to adenocarcinomas, the vacuoles in these cells contain acidic rather than neutral mucin, and the cytoplasm is cytokeratin negative (Figs. 17.17 and 17.18) [12].

Metaplasias

The fallopian tube is one of the least common sites showing epithelial metaplasia. Mucinous metaplasia is usually an incidental microscopic finding but may be associated with mucinous tumors of the cervix or ovary or with Peutz-Jeghers syndrome. Microscopically, there is often an abrupt transition from tubal epithelium to columnar cells with apical mucin vacuoles and basally located nuclei. They rarely show any

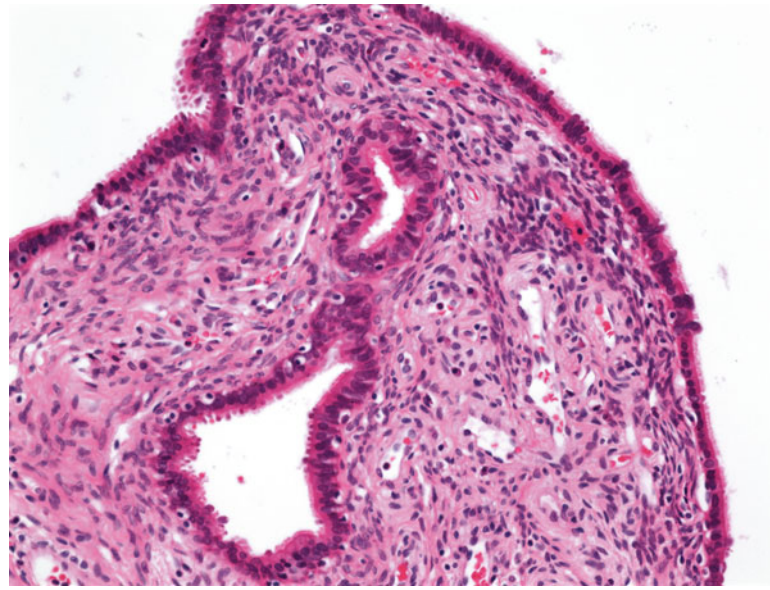

Fig. 17.15 Endometriosis. A diagnosis may be difficult to establish with routine light microscopy. The subepithelial hemorrhage and increased capillary proliferation may be subtle

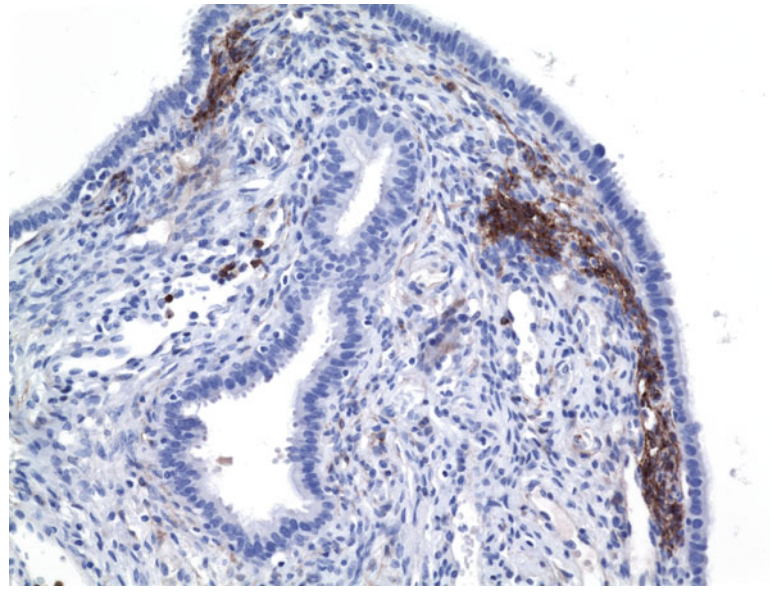

Fig. 17.16 Endometriosis (CD10 immunostain). Same location as in Fig. 17.15. A rim of endometrial-type stroma can be readily demonstrated with CD10 immunostain

extravasated mucin or cytologic atypia. In the presence of these, secondary involvement by or a coexistent mucinous neoplasm in the uterus or ovaries should be excluded first, as primary mucinous neoplasms, in particular, mucinous borderline tumors, are exceedingly rare [13–16].

Transitional cell metaplasia is usually an incidental and focal microscopic finding. It is characterized by serosal nests or plaques of transitional epithelium (Walthard nests), similar to those found in the mesosalpinx and mesovarium in women of all ages (Fig. 17.14). Those localized to the tuboperitoneal junction have been suggested to be precursors of some ovarian mucinous and Brenner tumors [17–19]. Others are the source of tubal transitional cell carcinomas [20, 21]. In addition, those found at the fimbriae may be misinterpreted as serous intraepithelial carcinomas. However, the cells in transitional cell metaplasia are cytologically bland and mitotically inactive. They lack p53 immunoexpression and the MIB-1 proliferative index is usually not

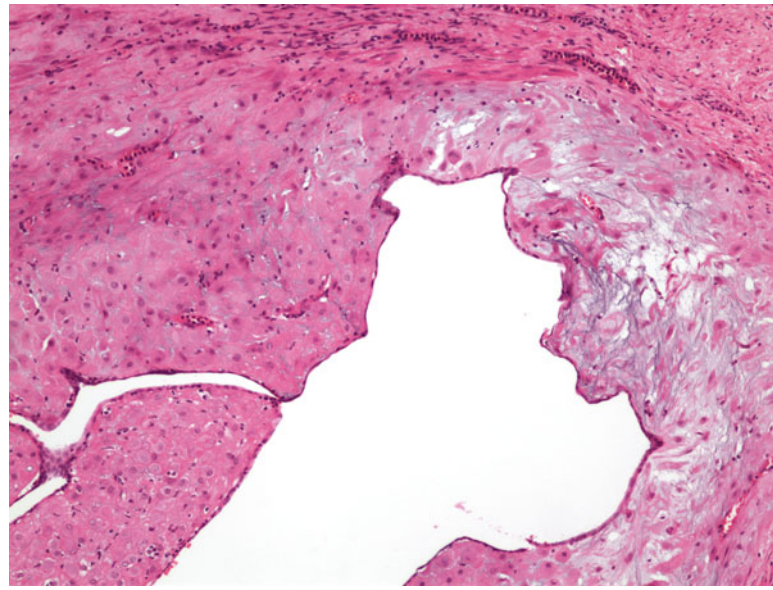

Fig. 17.17 Endometriosis. Predecidual change involving stromal cells in pregnancy or secondary to progesterone

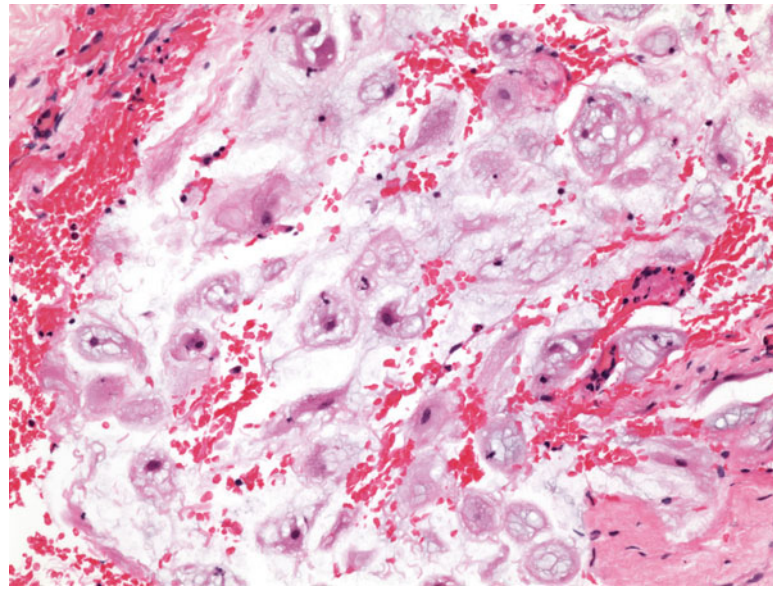

Fig. 17.18 Endometriosis. Signet-ring cell decidual change

increased [22]. Microscopically, the nests may undergo central cystification and may contain pale eosinophilic secretions. The epithelial cells usually have elongated nuclei with a nuclear groove (Fig. 17.19).

Squamous metaplasia is very rare and most likely the source of some very rare primary squamous cell carcinomas of the fallopian tube [23, 24].

Torsion

Torsion of the tube is usually associated with torsion of the ipsilateral ovary. It is uncommon as an isolated finding. Patients are usually in their reproductive age and present with acute abdominal pain. The affected tube is usually edematous and hyperemic and may be completely infarcted, depending on the duration and severity of the process [25, 26].

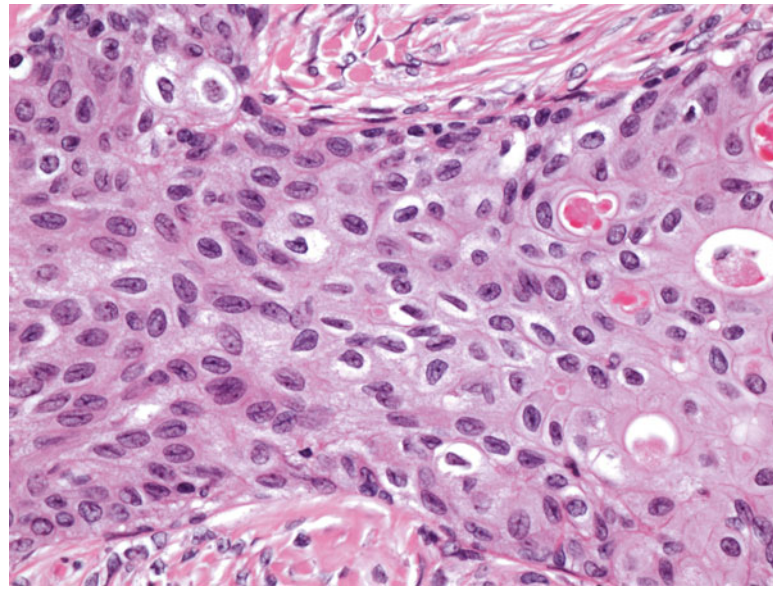

Fig. 17.19 Transitional cell metaplasia. The nuclei are typically elongated and there is often a nuclear groove. Note cystic change on right side

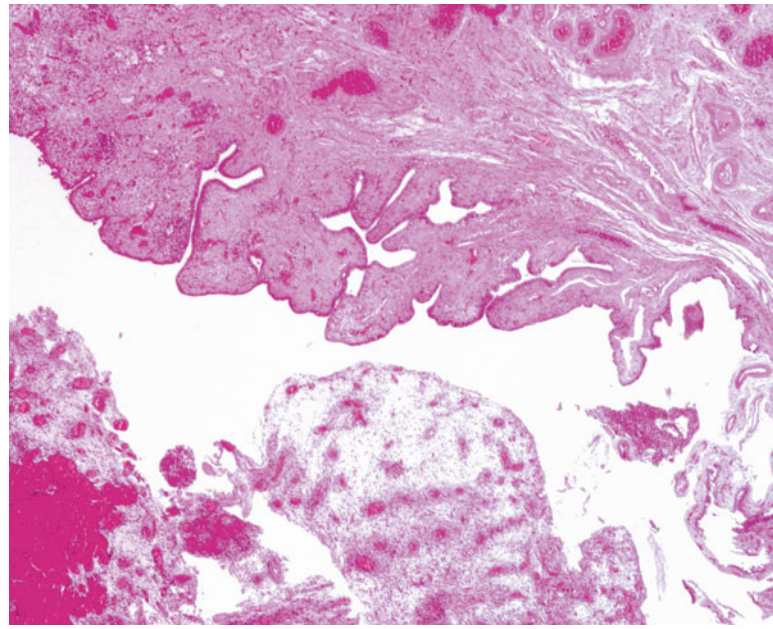

Fig. 17.20 Prolapsed fallopian tube. Tubal plicae may be found alongside pieces of granulation tissue in the vault

Calcification

Calcifications in the fallopian tube are usually of the dystrophic type and most often secondary to prior acute or chronic inflammation. When they are of psammomatous type, secondary involvement by an ovarian serous borderline tumor, or rarely one of primary tubal origin, should be excluded [27].

Prolapse

A "prolapsed" fallopian tube usually presents as a mass of granulation tissue in the vaginal vault in a patient who had a prior hysterectomy (Fig. 17.20). In >80 % of cases, it is a vaginal hysterectomy [28, 29]. This condition is usually a result of failure of closure of the peritoneum and may be related to usage of a postoperative drainage

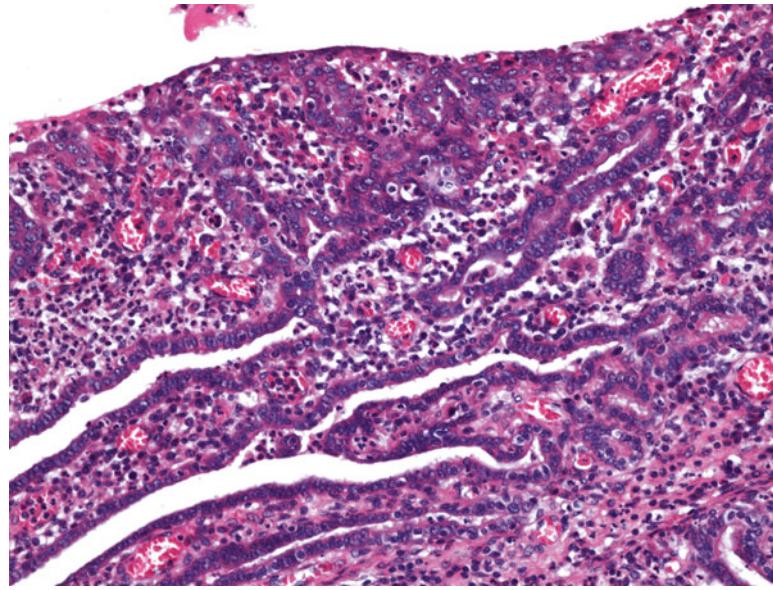

Fig. 17.21 Pseudocarcinomatous hyperplasia. There is glandular complexity and gland fusion in an inflammatory background

tube. The most important differential diagnosis is a papillary carcinoma [30]. The clinical history of a recent vaginal hysterectomy; identification of tubal plicae, with or without the muscular wall of the tube; and lack of significant cytologic atypia would favor a prolapsed tube. Rarely, the striking fibroblastic stromal reaction could give rise to a mass mimicking a mesenchymal neoplasm [31, 32].

Pseudocarcinomatous Hyperplasia

In contrast to carcinoma, pseudocarcinomatous hyperplasia is usually secondary to pelvic inflammatory disease and most commonly involves patients of reproductive ages (mean, 28.6 years). The more severe cases are associated with tuberculous or nontuberculous salpingitis. The lesion is often an incidental finding in patients undergoing treatment for other causes such as a leiomyoma or pelvic endometriosis. Grossly, the tube is enlarged and has a thickened wall but on sectioning, no tumor is identified. Microscopically, the pseudohyperplastic foci are usually small and may be multiple. The epithelium shows a papillary or cribriform pattern and may show pseudoinvasion of the underlying muscularis layer. There is no desmoplastic stromal reaction. The epithelial cells are stratified with cellular atypia and occasional mitoses. These changes are almost always accompanied by marked acute and chronic inflammation (Fig. 17.21). The subjacent mesothelial cells on the serosa may show reactive hyperplasia. The most important differential diagnosis is carcinoma. Features favoring pseudocarcinomatous hyperplasia include the absence of a tumor mass, the presence of coexisting chronic salpingitis, and the absence of atypical mitotic figures and desmoplastic stromal reaction [33, 34].

Pregnancy-Related and Trophoblastic Lesions

Ectopic Pregnancy

The fallopian tube accounts for >95 % of ectopic pregnancies. The majority (80 %) involves the ampulla, followed by the isthmus (10 %) and the infundibulum (5 %). Predispositions include a history of salpingitis, pelvic inflammatory disease, endometriosis, and tumors [35–37]. Tubal ectopic pregnancies usually present in the first 2 months with acute pain and abnormal bleeding. The diagnosis could usually be established with an elevated human chorionic gonadotropin (hCG) and ultrasound scan. Grossly, the tube is distended

with a congested serosa. Some cases may have already ruptured and with hemorrhage into the peritoneum. Cross section may reveal the presence of the white spongelike tissue or even the fetus (Fig. 17.22). Histologically, the appearances resemble those of intrauterine pregnancy, with chorionic villi filling the lumen and admixing with blood and fibrin, while trophoblasts are observed at the implantation site (Figs. 17.23 and 17.24). The trophoblasts may invade deep into the myosalpinx. Decidua is typically absent, unless there is coexisting endometriosis. In most instances, a predisposing cause can be found (Fig. 17.20). If chorionic villi are not identified in the initial histologic sections, it is important also to process additional tissue for microscopy as in some cases, chorionic villi may have degenerated or been lost due to rupturing of the tube. The diagnostic pitfall is a choriocarcinoma.

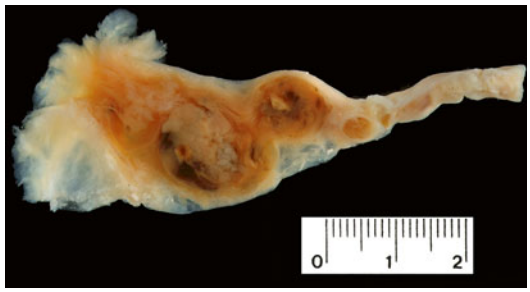

Fig. 17.22 Ectopic pregnancy. Spongelike tissue is seen filling and distending the tubal lumen associated with hemorrhage

Trophoblastic Lesions

Choriocarcinomas, complete and partial hydatidiform moles, and other trophoblastic lesions involving the fallopian tubes are very rare. The usual presentation is pain and abnormal bleeding, and in a minority of cases, ectopic pregnancy is suspected preoperatively.

Primary Tubal Choriocarcinomas

Primary tubal choriocarcinomas are exceptional, and metastasis from the more usual, intrauterine location should first be excluded [38–40]. The most common presentation is vaginal bleeding and an elevated serum hCG, or the patient may present with an ectopic pregnancy. Grossly, the tube is distended and the wall may have already been ruptured. Microscopically, the tubal lumen is usually filled with blood and sheets of trophoblasts without chorionic villi. The trophoblasts are evidently cytologically malignant on medium-power examination. They comprise an admixture of cytotrophoblasts and syncytiotrophoblasts arranged in a plexiform pattern.

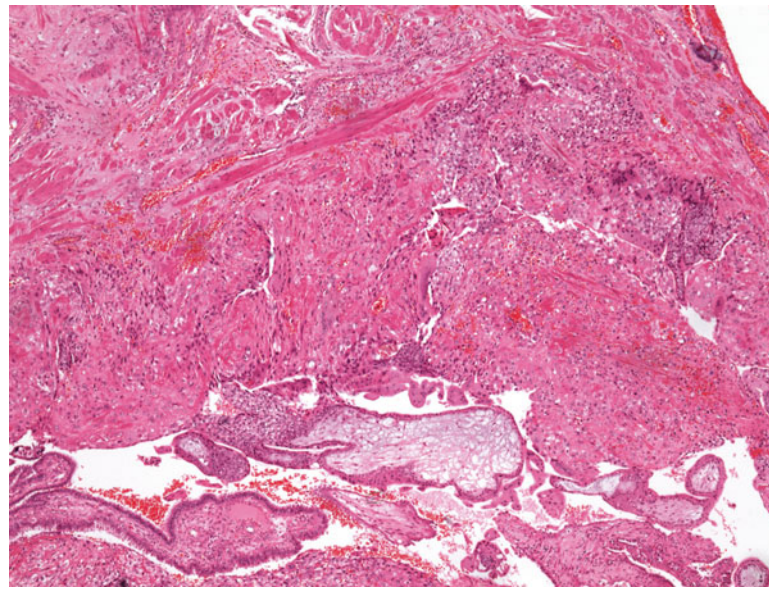

Fig. 17.23 Ectopic pregnancy

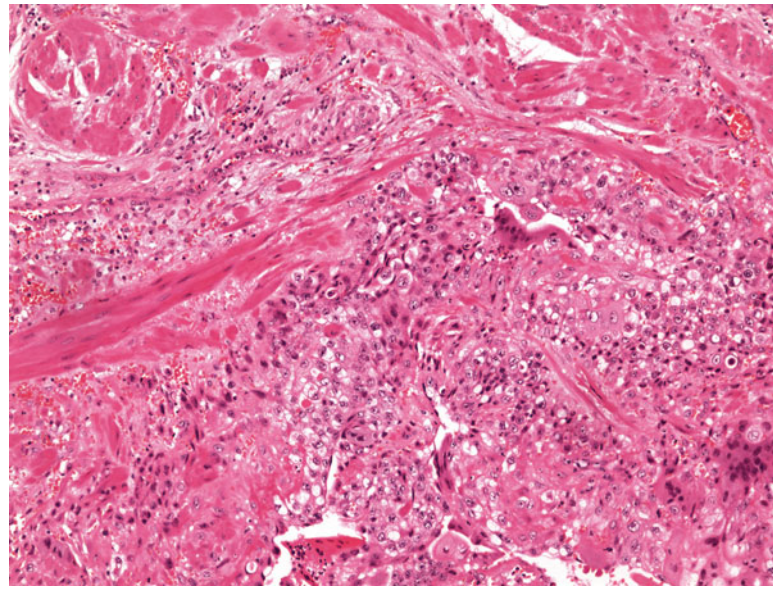

Fig. 17.24 Ectopic pregnancy. Implantation site trophoblasts invade the myosalpinx. Note absence of any decidua

Invasion of the myosalpinx is usually seen. Primary tubal choriocarcinomas may be both gestational and nongestational, and the latter may also be a component of a mixed germ cell tumor. Differential diagnosis between primary and metastatic and gestational and nongestational tumors would require additional clinicopathologic workup. After salpingectomy, the serum hCG in primary tubal choriocarcinoma usually falls. Patients with gestational choriocarcinoma may give a history of recent pregnancy while virginity would support a nongestational origin. Nongestational choriocarcinomas behave more aggressively and usually do not respond well to conventional chemotherapy used for treating gestational tumors, and it is therefore valuable to distinguish between the two. Molecular studies are usually required to confirm whether a tumor is gestational or nongestational. Microsatellite genotyping may be used in which the DNA of the patient and her partner and tumor and host tissue are used for comparison [41].

Hydatidiform Moles

Hydatidiform moles (HM) involving the tube are very rare, with only about 50 reported. In a large tertiary referral center in the United Kingdom, only eight cases of tubal HM were found among some 6,700 cases (0.1 %) over a thirteen-year period [40]. The majority of HMs are of partial type. It has been noted that the pathologic diagnosis of HM from the uterus is sometimes challenging and often requiring ancillary studies such as p57 immunohistochemical stain and molecular genotyping [42]. Diagnosis of tubal HMs is even more difficult as the numbers of chorionic villi are often less than that found in a uterine specimen. In tubal ectopic gestation, chorionic villi are more often hydropic due to degeneration; proliferation of extravillous trophoblast may be marked and usually shows invasion of the myosalpinx as part of the normal implantation site reaction (Fig. 17.25). Useful clues for confirming a nonmolar tubal gestation would include finding of polarized villous trophoblastic proliferation and absence of cytologic atypia in the extravillous, intermediate trophoblasts. Molecular studies may be required in unascertained cases. The risk of persistent gestational trophoblastic disease in tubal HMs is currently unknown.

Lesions of the Intermediate Trophoblasts

Lesions of intermediate trophoblast involving the fallopian tube include placental site nodule, placental site trophoblastic tumor and epithelioid trophoblastic tumor. To date, approximately 20

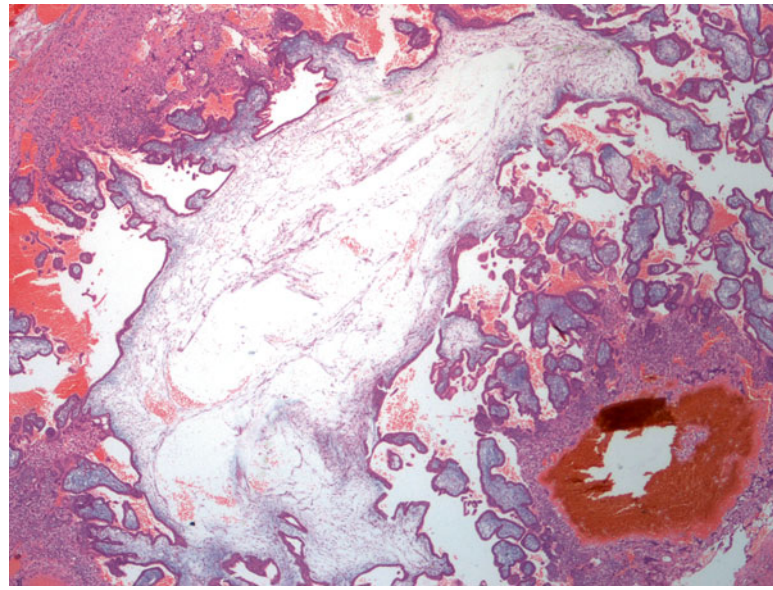

Fig. 17.25 Ectopic pregnancy. The limited number of chorionic villi in a tubal pregnancy showing hydropic change. Trophoblastic proliferation is marked but they remain polarized

of such cases have been reported [43–49]. In many of these, there was coexistent chronic salpingitis, endometriosis, or ectopic pregnancy.

Placental site nodule is usually related to a previous gestation that failed to involute completely. Apart from the usual intrauterine location, it has been described in the cervix but rarely in the tube. It is usually an incidental finding but patients may present with irregular menses or infertility. Grossly, the tube is usually normal or distended, and multiple nodules may be found on cross-sectioning. Microscopically, the trophoblastic cells have a degenerate or vacuolated appearance and are associated with marked stromal hyalinization. They are usually confined to the mucosa but occasionally, they may infiltrate the tubal wall. The cells are arranged in clusters or in isolation have eosinophilic to amphophilic cytoplasm and hyperchromatic nuclei but are mitotically inactive. Immunohistochemistry with hPL and hCG may be necessary in distinguishing these from non-trophoblastic tumors. The MIB-1 proliferative index is low.

Patients with tubal placental site trophoblastic tumor may present with irregular bleeding or infertility and may present with an ectopic pregnancy [44–48]. Grossly, the tube is distended with a hemorrhagic and friable mass. Most cases show cystic change. Microscopically, the cells are arranged in sheets and cords and have the propensity to infiltrate into vessel walls associated with deposition of fibrinoid material. There is often necrosis and hemorrhage. Most of the cells are mononuclear but occasionally there may be multinucleation. Mitoses, including atypical forms, may be seen. The tumor cells are immunoreactive for hPL, hCG, and cytokeratin. The MIB-1 proliferative index is usually high. Of the limited number of cases with follow-up, the clinical course has been benign.

A single case of epithelioid trophoblastic tumor has been reported and the patient has remained disease-free for 12 months after treatment with combination chemotherapy for high-risk gestational trophoblastic disease. The microscopic features are similar to tumors described in the uterus [49].

Nonneoplastic Alterations Related to Pregnancy

Arias-Stella reaction involving the fallopian tube may be seen in cases of ectopic tubal pregnancy and in occasional patients with an intrauterine pregnancy. The epithelial cells are usually hobnail with abundant eosinophilic or glycogen-rich

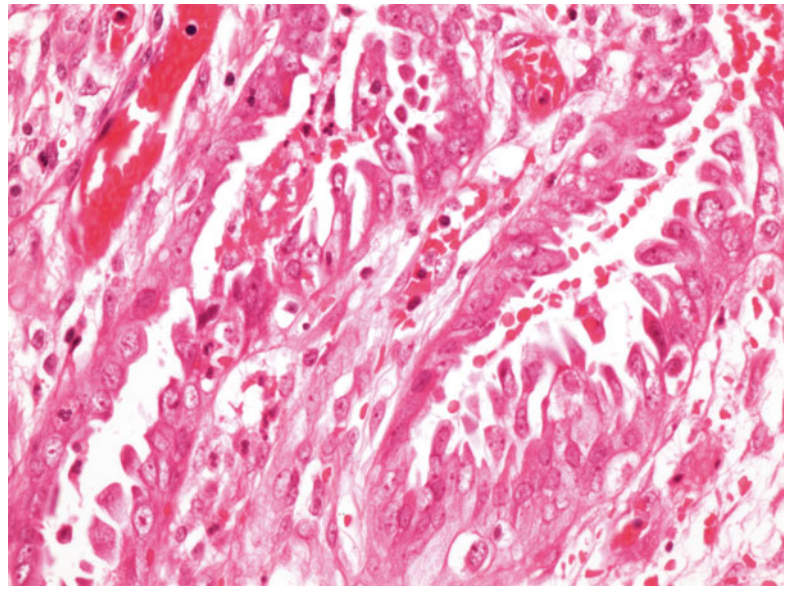

Fig. 17.26 Arias-Stella change. The tubal epithelial cells are hobnail, with cytoplasmic clearing and atypical nuclei with vesicular or smudged chromatin

vacuolated cytoplasm and highly atypical nuclei, which are usually markedly enlarged, irregular, and hyperchromatic. They may be pyknotic and sometimes with nuclear pseudoinclusions. Mitotic figures may be seen (Fig. 17.26). Rarely, the cellular changes are associated with glandular hyperplasia [50]. The most important differential diagnosis of Arias-Stella reaction is clear cell carcinoma. In contrast to the latter, the MIB-1 proliferative index is not elevated and the p53 immunostaining is either absent or weak and focal [51, 52].

Ectopic decidua may occasionally be found in the fallopian tubes in about one-third of ectopic pregnancies, in up to 8 % of normal-term pregnancy, and in about 5 % of tubes resected for treatment of contraception but rarely in women on progestogens [53–55]. The decidualized cells are negative for cytokeratins and immunoreactive for CD10. The cells may sometimes undergo signet-ring cell change and may potentially mimic an adenocarcinoma.

Benign Tumors

Metaplastic Papillary Tumor

Metaplastic papillary tumor of the tube is a rare lesion and is usually found incidentally in tubal specimens resected during the postpartum period. It rarely occurs in nonpregnant women. It has rarely been studied in detail but has always been considered a metaplastic, rather than neoplastic lesion [56, 57]. A recent study using microsatellite analysis, however, suggested that this lesion may in fact be a tumor that is closely related to ovarian serous borderline tumors [58]. Microscopically, there is morphologic resemblance to ovarian serous borderline tumor, characterized by intraluminal proliferation of papillae covered by müllerian-type epithelial cells with abundant eosinophilic cytoplasm and occasional mucous cells. Rare mitotic figures may be present.

Endometrial Polyp

The endometrial polyp is a common cause of tubal obstruction, ectopic pregnancy, and infertility [7, 59, 60]. They are most often bilateral and involve the interstitial portion of the tubes, where there is frequently an extension of endometrial mucosal lining from the corpus. Histologically, they are identical to the uterine counterpart.

Hyperplasia

Although the diagnostic criteria for tubal hyperplasia are variable and not well established, some cases, in particular those that are multifocal or

comprise small rounded clusters of epithelial cells, have been implicated as the putative precursor of ovarian serous borderline tumors. Some of these are associated with the presence of psammoma bodies [34, 61, 62]. It should be noted, however, that mild proliferation of the tubal epithelium is common and may be related to unopposed estrogenic stimulation. Others may also be reactive and secondary to inflammation.

Borderline Tumors

Serous borderline tumors of the Fallopian tube are very rare but have been described within the fimbrial ends [20, 63]. They are usually found to occur in reproductive-aged women and are usually incidental findings. Grossly, they are small, with a mean size of 1.8 cm. The histologic appearances are identical to that described for serous borderline tumors of the ovary. If the lesion involves a fallopian tube that is resected in a pregnant patient, a metaplastic papillary tumor has to be considered. However, these two lesions may share an overlapping morphology, and distinguishing between the two may be difficult. The most important differential diagnosis is from tubal serous carcinoma. The typical hierarchical branching papillae of borderline tumors, the absence of highly stratified nuclei, slit-like spaces between cells, and lack of high-grade nuclear features are more compatible with a borderline tumor [64]. The few reported cases of tubal serous borderline tumors appear to behave in a benign fashion [65, 66]. Several mucinous borderline tumors of the tube have been reported which may have arisen from metaplastic mucinous epithelium; others were found in association with pseudomyxoma peritonei, but these may have represented metastases from undetected low-grade appendiceal mucinous neoplasms [13].

Malignant Tumors

Carcinomas

General Features

Fallopian tube carcinomas are uncommon and account for <1 % of malignant tumors of the female genital tract, but the true frequency is probably higher because carcinomas involving both the tube and ovary are generally considered as ovarian in origin [67, 68]. Tumors related to BRCA gene mutations are discussed in the next section.

Clinical Features

Most patients are postmenopausal (mean, 57 years) and present with postmenopausal bleeding, which is rarely associated with pain and/or a mass. Ascites at presentation has been noted in 5–14 % of patients [69, 70]. In less than 10 % of the cases, there is "hydrops tubae profluens" in which a watery vaginal discharge is followed by the relief in colicky pain and a decrease in the size of an abdominal mass [71]. If the tumor fragments are dislodged, they may be discovered in cervical cytology or endometrial curettings, but in these cases, a uterine primary is usually first suspected [72]. Exceptionally, the first presentation is lymphadenopathy [73]. The serum CA-125 is usually high and occasionally, there is ectopic production of β-HCG. A fallopian tube carcinoma rarely gives rise to a paraneoplastic syndrome [74–76]. Tumors discovered in cases of prophylactic bilateral salpingo-oophorectomy in those associated with BRCA mutations are usually asymptomatic.

Gross Features

The overwhelming majority of tumors are unilateral, with 3–13 % being bilateral [20, 21, 69, 77, 78]. Most are markedly dilated and may appear as a hydrosalpinx, hematosalpinx, or pyosalpinx. The serosa is either smooth and hemorrhagic or granular. On sectioning, one or more solid tumor nodules of a mean size of 4.5 cm may be found in the wall, with or without papillary tissue projecting into the lumen. There is often hemorrhage and necrosis. More than 92 % of tumors are located at the distal two-thirds of the tube, and only 8 % are at the fimbriated end [20]. In cases where the tumor adheres to and involves the ovary, distinction from an ovarian primary with secondary tubal involvement may be impossible. It has been suggested that these cases should be referred to as "tubo-ovarian carcinomas."

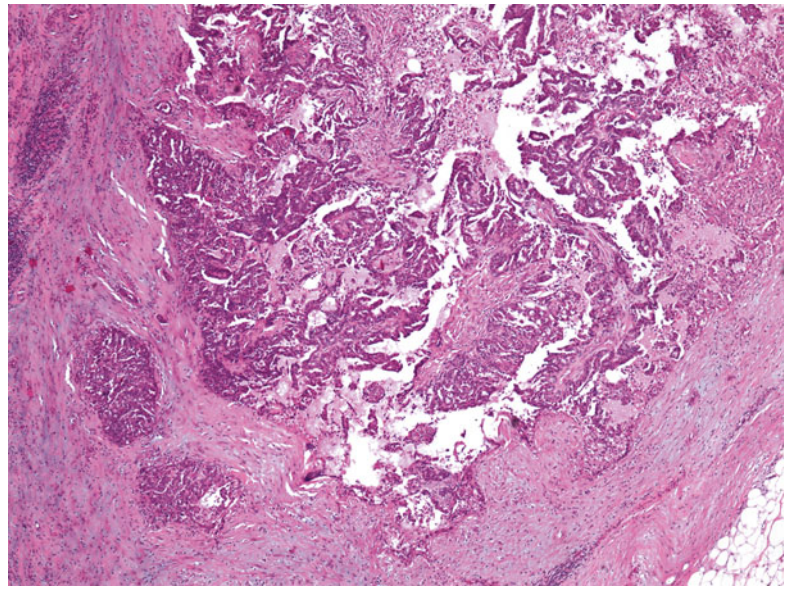

Fig. 17.27 Primary tubal serous carcinoma. Stage Ia-2

Microscopic Features

More than 50 % of tumors are serous carcinomas; 25 % are endometrioid; the remainder is an admixture of transitional cell carcinomas and other cell types [20, 21].

Serous Carcinomas

These are identical to those of the ovaries or peritoneum, with papillae projecting into the lumen, or glands or solid islands composed of stratified tumor cells with slit-like spaces. The tumor cells are said to have lost the cilia and acquired secretory cell differentiation (Figs. 17.27, 17.28, 17.29, and 17.30) [79]. Psammoma bodies may be seen.

Small and noninvasive serous carcinomas are usually papillary and had been termed "carcinoma in situ." This is an outmoded terminology as it implied the lesion was not capable of spreading. On the contrary, the cells of noninvasive and small serous carcinomas may shed, dislodge, and spread beyond the fallopian tube, commonly from the open fimbrial end to the peritoneum. The more appropriate and now acceptable term is "tubal intraepithelial carcinoma."

Serous tubal intraepithelial carcinoma (STIC) is now recognized as the precursor of some serous carcinoma (Figs. 17.31, 17.32, and 17.33) [80, 81]. In its earliest form, including in those cases in which the tubes are removed prophylactically in BRCA mutation carriers, detection may difficult.

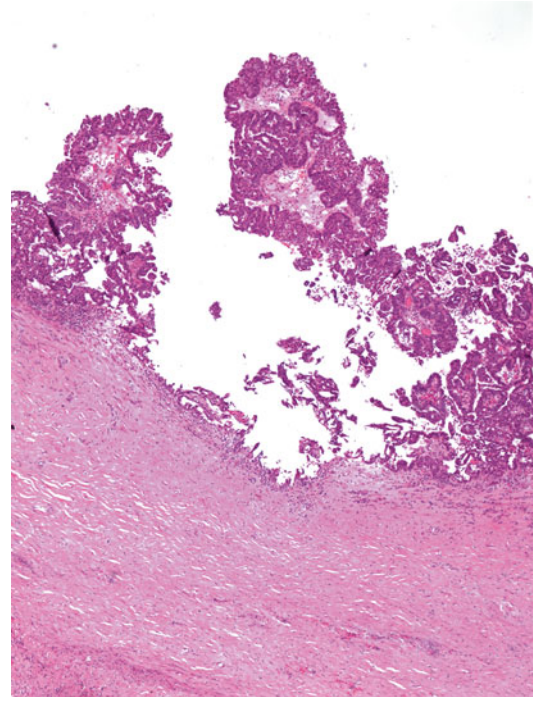

Fig. 17.28 Primary tubal serous carcinoma. Stage Ia-0

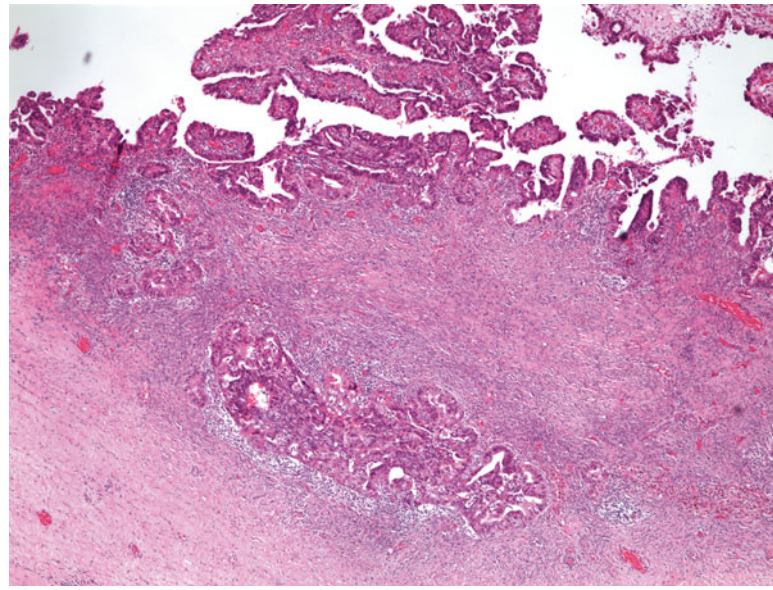

Fig. 17.29 Primary tubal serous carcinoma. Invasion of muscularis

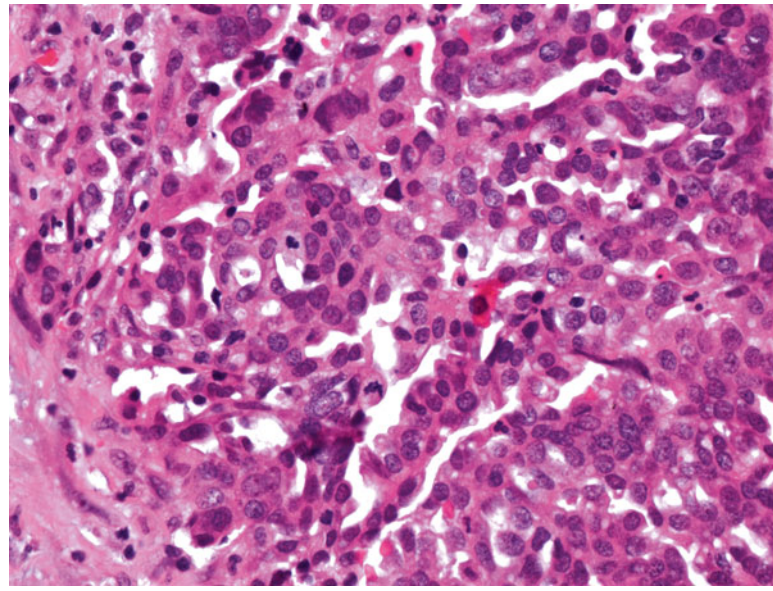

Fig. 17.30 Primary tubal serous carcinoma. The tumor cells are stratified, with slit-like spaces between groups of cells. They have grade 3 nuclear features and brisk mitotic activity

The distinction between normal and reactive atypia and/or STIC is challenging if morphology is used alone [82]. The most agreeable features for STIC among experienced gynecological pathologists in one study included the presence of >1 mitotic figure and nuclear stratification. Immunohistochemistry is crucial in the diagnosis of STIC. The MIB-1 proliferative index is usually elevated in STIC and not in nonneoplastic tubal epithelium [83]. The p53 immunostain either shows diffuse moderate-to-strong staining or may be completely negative, due to a missense or nonsense TP53 mutation [84–86]. It is important to note however, before a diagnosis of STIC is made, the entire fallopian tube should be processed for histologic evaluation as to exclude a nearby and/or small invasive serous carcinoma. STICs have also been shown to coexist with similar serous intraepithelial carcinomas involving the endometrium [87].

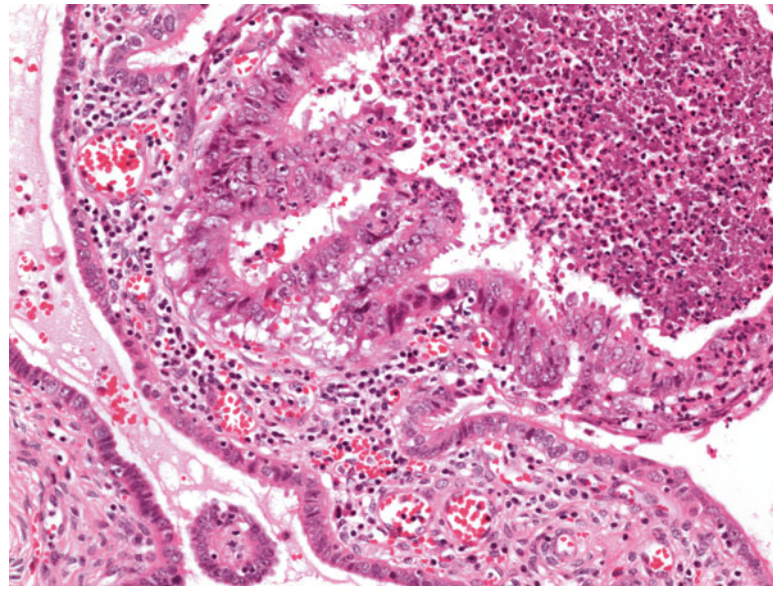

Fig. 17.31 Serous tubal intraepithelial carcinoma. The lesion may be localized and small. Note adjacent normal epithelium

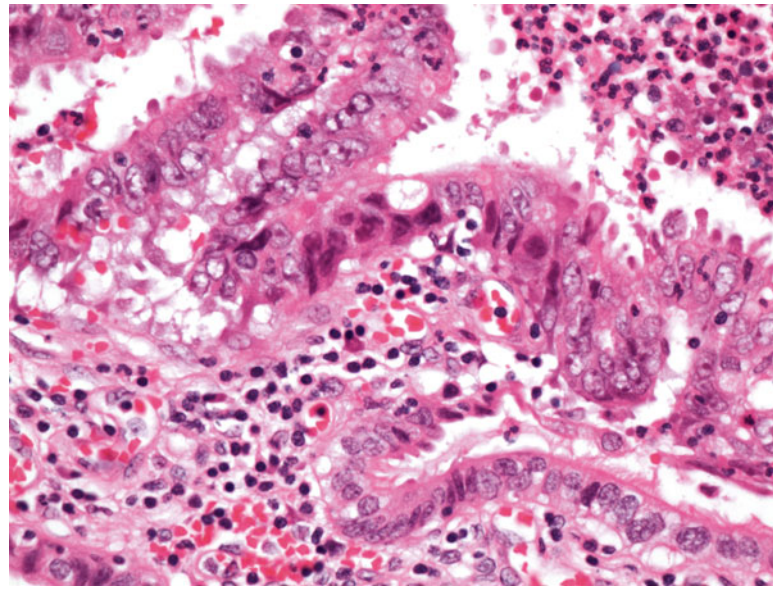

Fig. 17.32 Serous tubal intraepithelial carcinoma. The tumor cells are stratified, with variation in nuclear size, vesicular nuclear chromatin and prominent nucleoli, in contrast to adjacent, nonneoplastic epithelium. Note the absence of cilia over the luminal border of the tumor cells

Secretory cell outgrowths (SCOUTs) in the fallopian tube, characterized by loss of PAX2 staining immunohistochemically, have also been implicated as a precursor to high-grade serous carcinomas [88]. Some cases may also be precursors to ovarian serous borderline tumors [89].

Endometrioid Carcinomas

The second most common primary tubal carcinoma also resembles its uterine counterpart (Fig. 17.34). Some are related to endometriosis. Although the majority is usually noninvasive or only superficially invasive, more than 80 % are

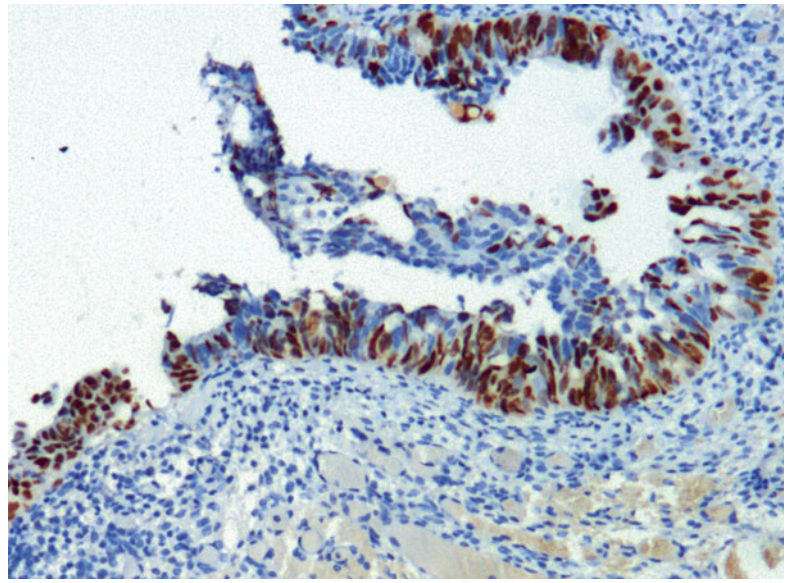

Fig. 17.33 Serous tubal intraepithelial carcinoma. P53 immunostain

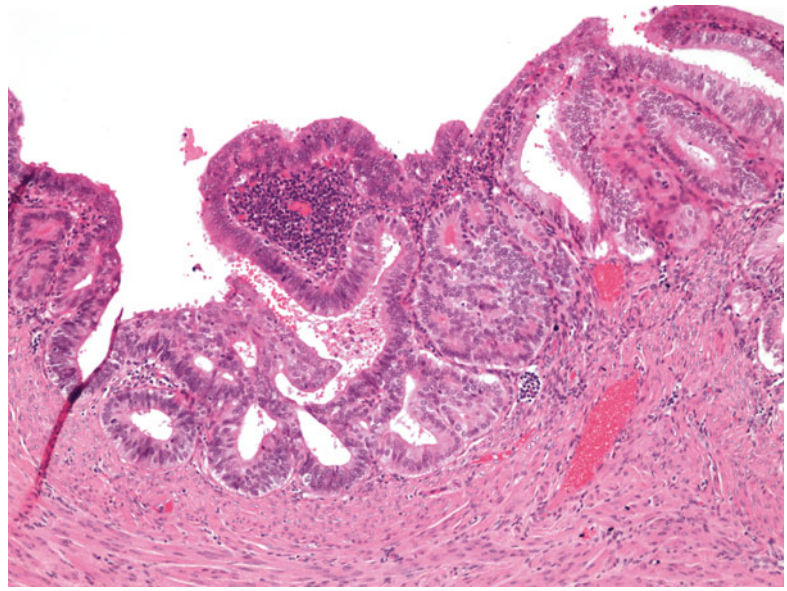

Fig. 17.34 Endometrioid carcinoma. Stage Ia-0

usually of FIGO grade 2 or 3 [20, 21, 90]. Similarly, for endometrioid tumors of other sites, morphologic variants are common, such as squamous differentiation, spindle cell differentiation (solid proliferation of epithelial spindle cells), microglandular hyperplasia-like, sex cord-like, trabecular, and even oxyphilic type [20, 21, 90]. In some cases, independent primary endometrioid carcinomas may arise in the tube and in the corpus [91].

Other Cell Types

Primary transitional cell carcinomas most likely arise from metaplastic epithelium [17], but many cases are probably mixed tumors, especially of serous differentiation [92, 93]. Mucinous carcinomas are uncommon and must be distinguished from metastatic carcinoma from other sites, such as the endocervix [94]. Other uncommon

subtypes include undifferentiated carcinoma, clear cell, squamous cell, lymphoepithelioma-like, and hepatoid, and glassy cell carcinomas of the tube have also been reported [20, 21, 95–97].

Prognosis

The most important prognostic factor is the stage. In several series, in 21–56 % the tumors were stage I, 9–20 % stage II, 16–55 % stage III, and 4–12 % stage IV [20, 21, 69, 78, 98–103]. Tubal carcinoma spreads most commonly to the peritoneum and adjacent organs. At presentation, lymph node involvement is frequent, most commonly para-aortic and pelvic lymph nodes, and occasionally, inguinal lymph nodes. Nodal metastases have been detected even in cases where the tumors were only confined to the tube [20, 69, 104–107].

In 1999, the staging system of International Federation of Gynecology and Obstetrics (FIGO) was modified in order to take noninvasive carcinomas of the fallopian tube into consideration (Table 17.1). It was proposed that stage Ia tumors should be substaged into Ia-0 (intramucosal with no lamina propria invasion), Ia-1 (invasion only of lamina propria), and Ia-2 (deeper invasion to muscularis layer but no serosal involvement). This change reflected the fact that there was a decreasing survival with increasing depth of invasion. The 5-year survival in the largest study

Table 17.1 Modified FIGO staging for fallopian tube carcinoma [21]

Stage 0	Carcinoma in situ (limited to tubal epithelium)
Stage I	Growth limited to tube
Stage Ia	Growth limited to one tube without extension through or onto serosa, ascites containing malignant cells, or positive peritoneal washings
Stage Ia-0	Growth limited to one tube with no extension into lamina propria
Stage Ia-1	Growth limited to one tube with extension into lamina propria but no extension into muscularis
Stage Ia-2	Growth limited to one tube with extension into muscularis
Stage Ib	Growth limited to both tubes without extension through or onto serosa, ascites containing malignant cells or positive peritoneal washings
Stage Ib-0	Growth limited to both tubes with no extension into lamina propria
Stage Ib-1	Growth limited to both tubes with extension into lamina propria, but with no extension into muscularis
Stage Ib-2	Growth limited to both tubes with extension into muscularis
Stage Ic	Tumor either stage Ia or Ib but with extension through or onto tubal serosa or with ascites
Stage I(F)	Tumor limited to fimbriated end of tube(s) without invasion of tubal wall
Stage II	Tumor involving one or both fallopian tubes with pelvic extension
Stage IIa	Extension and/or metastasis to uterus and/or ovaries
Stage IIb	Extension to other pelvic tissues
Stage IIc	Tumor either stage IIa or IIb with ascites containing malignant cells or with positive peritoneal washings
Stage III	Tumor involving one or both fallopian tubes with peritoneal implants outside pelvis, including superficial liver metastases, and/or positive retroperitoneal or inguinal nodes. Tumor limited to pelvis except for histologically proved extension to small bowel or omentum
Stage IIIa	Tumor grossly limited to pelvis with negative nodes but with histologically confirmed microscopic seeding or abdominal peritoneal surfaces
Stage IIIb	Tumor involving one or both fallopian tubes with grossly visible, histologically confirmed implants of abdominal peritoneal surfaces, none exceeding 2 cm in diameter. Lymph nodes are negative
Stage IIIc	Abdominal implants >2 cm in diameter and/or positive retroperitoneal or inguinal nodes
Stage IV	Growth involving one or both fallopian tubes with distant metastases including parenchymal liver metastases. If pleural effusion is present, fluid must be positive cytologically for malignant cells

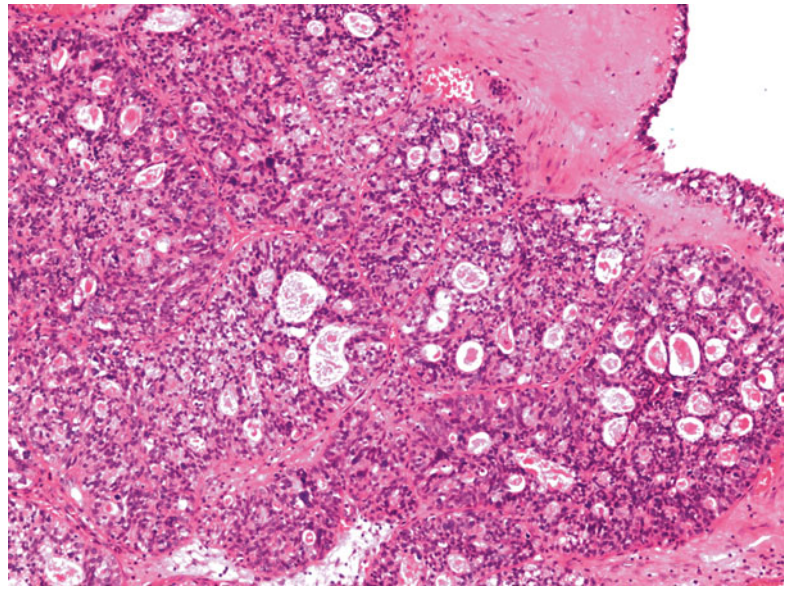

Fig. 17.35 Endometrioid carcinoma. This tubular pattern may be confused with a female adnexal tumor of probable wolffian origin

for stage 0, I, II, III, and IV was 88, 73, 37, 29, and 12 %, respectively [20, 69]. Stage 0, represented by TIC, is no longer listed in the current FIGO system. It has been noted that even for stage 0, the tumor may be dislodged and spread beyond the fallopian tube. This is particularly important for those arising from the fimbriated end where tumors without invasion can have direct access to the peritoneal cavity and may behave more aggressively than tumors located more proximally. Tumors arising from the tubal fimbriae are now considered the origin of some pelvic and peritoneal serous carcinomas [108, 109]; they are designated stage I(F) [20, 21, 110].

Other adverse prognostic factors have included increasing age, a patent tubal ostium, vascular invasion, and a high volume of residual tumor. Histologic subtype has not been shown to be of prognostic significance [21, 69, 98, 101].

Differential Diagnosis

A significant proportion of tumors of endometrioid differentiation had areas resembling a female adnexal tumor of probable wolffian origin (FATWO), potentially a cause of diagnostic confusion. In these, there is an intraluminal solid mass of tumor cells punctured by numerous small glands, creating a sievelike appearance (Fig. 17.35) [21]. In contrast to FATWOs, tubal endometrioid carcinomas are intraluminal tumors and usually contain areas more typical of endometrioid carcinomas, as well as areas of squamous differentiation and true glands formation. Endometrioid carcinoma with spindle cell differentiation may be confused with malignant müllerian mixed tumor (Fig. 17.36).

Carcinomas must be distinguished from pseudocarcinomatous hyperplasia.

BRCA-Related Tubal Carcinomas

Patients with BRCA mutations are more susceptible to develop breast and ovarian cancers. The risk for tubal carcinoma appears to be even greater than that for ovarian carcinoma; and the risk appears to begin early in the fifth decade. The proportion of tubal carcinomas that are BRCA-related has varied from 16 to 43 % [111–113]. For carriers of germ line mutation for BRCA1

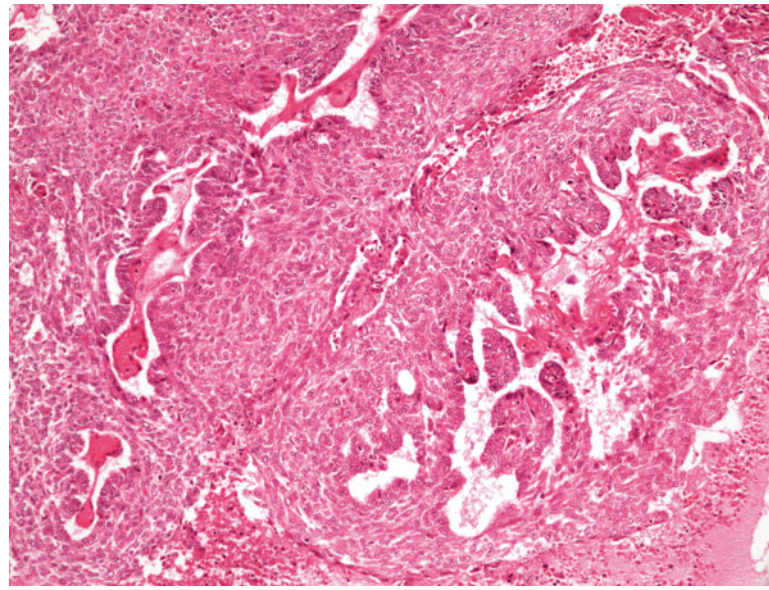

Fig. 17.36 Endometrioid carcinoma. Spindle cell differentiation may be confused with a malignant müllerian mixed tumor

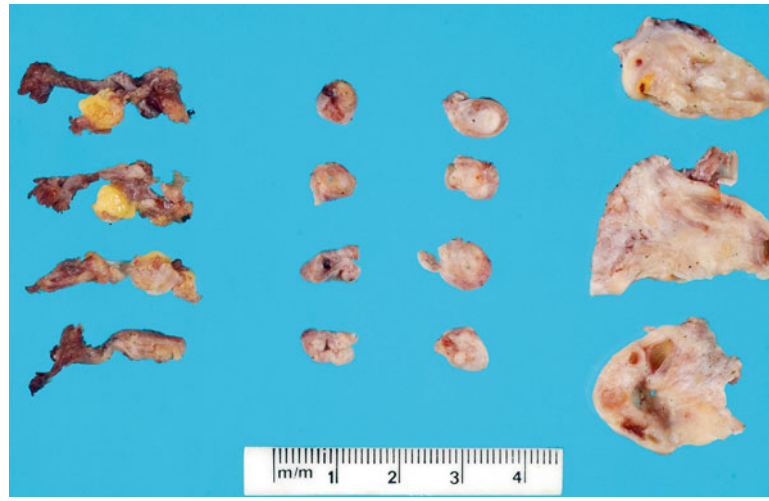

Fig. 17.37 Following the SEE-FIM protocol to maximize the proportion of fallopian tube mucosa for microscopic examination. The tubes and ovaries are completely processed for microscopy. The tubal infundibulum is amputated and sectioned longitudinally (*left*) in order to maximize the exposure of fimbrial mucosa. The ampullary portion (*middle*) is cut serially at cross section at 2–3 mm intervals

and BRCA2 genes, risk-reducing salpingo-oophorectomy by age 40 has become an accepted treatment. This approach has led to the recognition of clinically occult serous tumors in 5 % of resected tubes. Most of these were either EIC or superficially invasive and in about 80 %, they are found in the fimbriae [114–119]. Tumors arising from this region are extremely small and would have been less commonly recognized by the usual practice of selective pathologic sampling of the fallopian tube. Given the importance of complete histologic sampling of the fallopian tubes and ovaries in these high-risk patients, a protocol for "sectioning and extensively examining the fimbrial end" (SEE-FIM) has been proposed [120] (Fig. 17.37) (Table 17.2).

Table 17.2 The SEE-FIM protocol for examining fallopian tubes in prophylactic salpingo-oophorectomies [120]

1.	Tubes and ovaries are fixed in formalin for 1–2 hours to reduce the risk of exfoliation during sectioning
2.	The distal 2 cm of the tube are amputated and sectioned sagittally into 4 sections
3.	The remainder of the tubes are sectioned at 2- to 3-mm intervals
4.	The ovaries are sectioned at 2- to 3-mm intervals
5.	The entire tube and ovary are submitted for histologic analysis
6.	Areas of mucosa containing discrete loss of cilia with atypia are immunostained for both p53 and MIB-1 at the discretion of the pathologist
7.	In the event that special stains are used to verify a diagnosis of intramucosal carcinoma, the diagnosis must be corroborated by a second observer before issuing a pathology report

Tumors of Mixed Epithelial-Stromal Origin, Mesenchymal Tumors, and Metastatic Tumors

Adenomatoid Tumor

The adenomatoid tumor is the commonest benign tumor of the fallopian tube and is usually an incidental finding. Similar identical tumors have been described in the uterus, pouch of Douglas, or ovary with which the tubal tumor may coexist. Their cell of origin is presumed to be mesothelial [121]. Grossly, the tubal lesion is usually small but may be up to 2 cm in size appearing as a white to yellow mural nodule. Occasionally they are bilateral. Microscopically, multiple cystically dilated tubules or compressed slit-like spaces are distributed throughout a hyperplastic smooth muscle stroma, creating a pseudoinvasive pattern. Sometimes, the tubules may be closely packed and with limited amount of intervening smooth muscle. The tubules or spaces are lined by an attenuated layer of cuboidal cells with eosinophilic cytoplasm. Cytologic atypia may be mild and is usually secondary to infarction but there are usually no mitotic figures (Figs. 17.38 and 17.39) [122]. The most important differential diagnosis is metastatic signet-ring cell adenocarcinoma. A signet-ring cell adenocarcinoma would normally stain with a mucicarmine stain and be immunoreactive for epithelial markers but not for mesothelial cell markers. The cystically dilated tubules in some adenomatoid tumors may be confused with a lymphangioma; the latter may be excluded with negative immunoreactivity for endothelial cell markers, such as CD31 or factor VIII-related antigen.

Adenofibroma and Adenosarcoma

Morphologically, tubal adenofibromas resemble those encountered in the ovary and uterus. Tumors of tubal origin are rare and are usually an incidental finding but may occasionally be the cause of an ectopic pregnancy. They have also been found in tubes resected in patients with BRCA gene mutations [123–125]. Adenofibromas are usually <1 cm in size or are only discovered microscopically. Adenosarcomas are exceptional, including one of fimbrial origin that recurred [126]. It is conceivable that the behavior of these tumors would be dependent on the presence or absence of sarcomatous overgrowth, as in the ovarian and uterine counterparts.

Malignant Müllerian Mixed Tumor

There are approximately 50 reported cases of malignant müllerian mixed tumors (MMMTs) with most being case reports. They account for <5 % of all primary tubal malignancies [127]. They are less common than similar tumors involving the ovaries or uterus [128–130]. Women affected are usually postmenopausal. Although they may present with a watery or bloody vaginal discharge, abdominal pain, or an adnexal mass, a tubal origin for the tumor is rarely made preoperatively. At the time of diagnosis, most tumors have spread beyond the tube. Gross examination usually shows a tumor with a variegated cut surface filling the lumen. Fimbrial

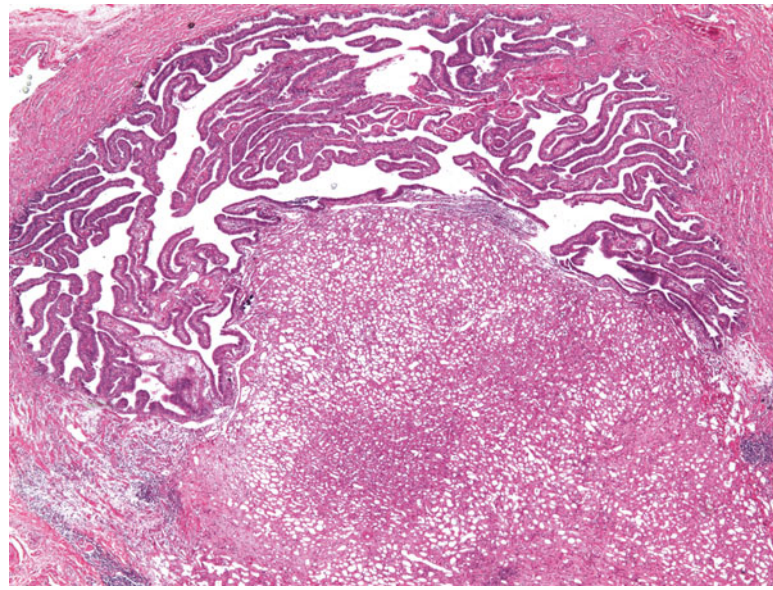

Fig. 17.38 Adenomatoid tumor. Cystically dilated, closely packed tubules compress the tubal mucosa with narrowing of its lumen

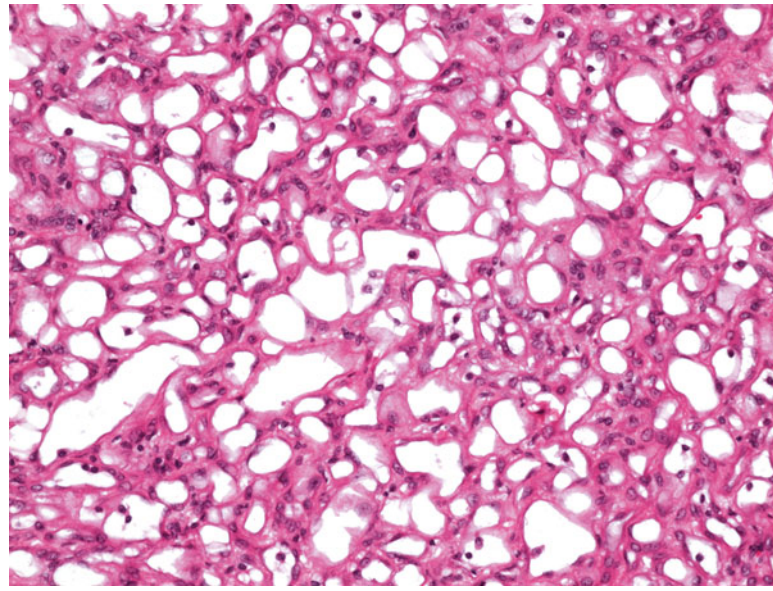

Fig. 17.39 Adenomatoid tumor. The tubules are lined by an attenuated layer of bland epithelial cells without cytologic atypia

origin has been described [131]. Microscopic features are identical to MMMTs found elsewhere. Coexisting TIC has been reported [128, 131]. In more than 50 % of reported cases, the tumors contained heterologous elements (Figs. 17.40 and 17.41). To diagnose a tubal MMMT, a normal ovary should be identified. When there is involvement of both the tube and ovary, the site of origin cannot be ascertained. MMMTs of the tube are aggressive but survival may be prolonged if disease is confined to the pelvis, when all visible tumor is completely resected and when there is no ascites [128]. The most important differential diagnosis is endometrioid carcinoma with spindle cell differentiation. In contrast to MMMT, the spindle cells in this tumor are usually of lower nuclear grade and merge with the glands.

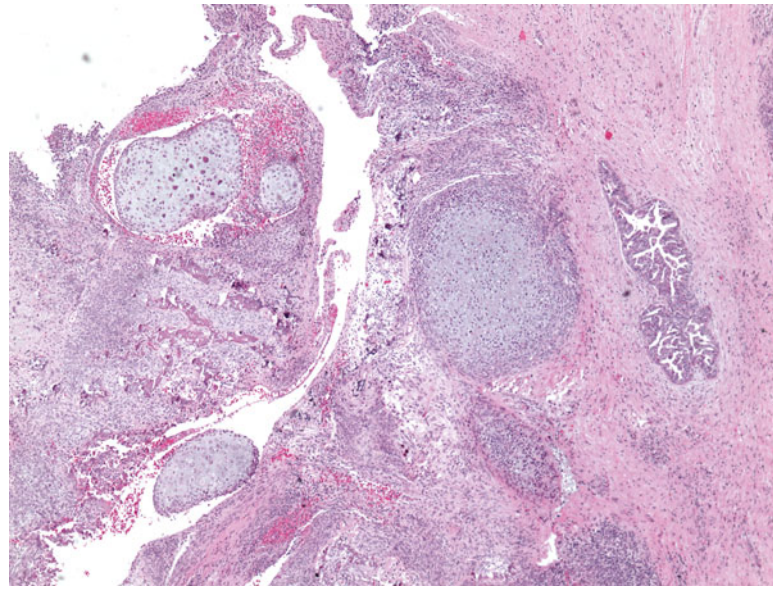

Fig. 17.40 Malignant müllerian mixed tumor. Both carcinomatous and sarcomatous components are infiltrating the tubal wall, while the latter also projects into the tubal lumen

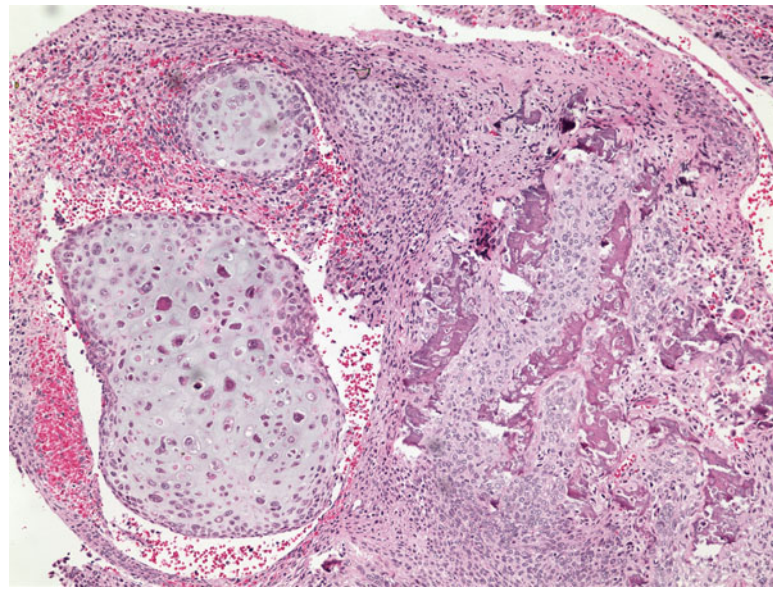

Fig. 17.41 Malignant müllerian mixed tumor. The sarcomatous component is an admixture of malignant osteoid and cartilage, surrounded by undifferentiated spindle cells

Leiomyoma

Leiomyoma is the commonest mesenchymal tumor of the fallopian tube even though rare. These tumors are usually asymptomatic or detected incidentally during operations for other reasons. They may occasionally be discovered during investigation for infertility or may cause ectopic pregnancy [132–134]. Microscopically, they are identical to the uterine counterpart and may be subject to similar degenerative changes and cause diagnostic difficulties.

Sarcomas

Primary tubal sarcomas are rare. Among them, the majority are leiomyosarcomas [135]. The rest include embryonal rhabdomyosarcoma, malignant fibrous histiocytoma, and synovial sarcoma

[136–138]. Leiomyosarcomas have the same presentation as tubal carcinomas and do not differ from uterine leiomyosarcomas grossly, microscopically, or biologically. The most important differential diagnosis to consider is metastatic gastrointestinal stromal tumor [139]. A panel of immunostains to exclude this should include C-KIT, DOG-1, and CD34.

Metastatic Tumors

Involvement of the tube by metastases or by direct extension from the ovary or endometrium is more common than a primary tubal carcinoma [140]. Although most metastatic carcinomas usually involve the serosa, serous carcinomas of ovarian origin may be found in the lumen but without invasion to the wall [141]. This should be distinguished from a focus of EIC. Direct extension by ovarian serous borderline tumor is not uncommon, and the appearance usually resembles a peritoneal noninvasive implant. Rarely would a cervical squamous carcinoma show pagetoid-like involvement of the tube [142]. Uterine endometrial stromal sarcomas may infiltrate into the tube, either directly or through vascular channels [143].

Paratubal Lesions

Paratubal Cysts

Paratubal cysts are common and usually an incidental finding. Larger cysts may result in torsion of the appendage and patients may present with an acute abdomen. They range from microscopic to very large and may be confused with an ovarian serous cystadenoma or hydrosalpinx (Fig. 17.42). The cysts may be of paramesonephric (müllerian), mesothelial, or mesonephric (wolffian) origin, with the first being the commonest, and are referred to as hydatids of Morgagni [144]. They are usually found near the tubal fimbria and lined by tubal-type epithelium that may form folds resembling the tubal plicae.

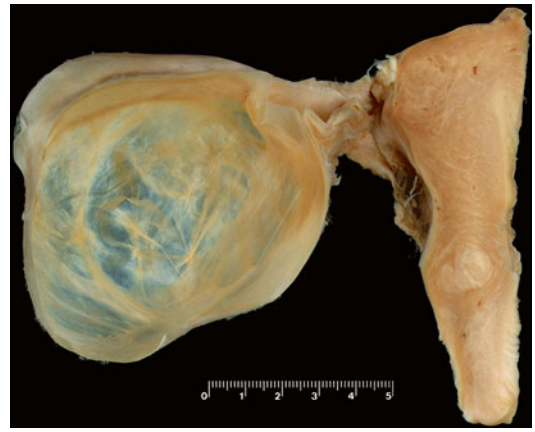

Fig. 17.42 Paratubal cyst. The wall is usually thin and translucent. It may become large and mimic an ovarian cyst

In some cases, the epithelium is completely attenuated and distinction between different types of cysts is not possible. Mesonephric cysts are lined by cuboidal, usually nonciliated cells and usually have a muscular wall. Paratubal cysts are benign.

Female and Adnexal Tumor of Probable Wolffian Origin

Female adnexal tumor of probable wolffian origin (FATWO) is a rare tumor and its origin is assumed to be the mesonephric remnants within the leaves of the broad ligament [145–149]. Rarely may they be found in the ovary or peritoneum. They have been reported in women from 15 through 72 years (mean, 46) who usually present with pain and/or a mass. The tumors are unilateral with a mean size of 8 cm. They are circumscribed, firm, rubbery, and white, either involving the leaves of the broad ligament or attached to the fallopian tube by a pedicle. There may be cystic change but hemorrhage or necrosis is rare. Microscopically, the combination of solid, cystic, and tubular growth patterns creates a sievelike appearance with prominence of luminal eosinophilic secretions (Figs. 17.43, 17.44, and 17.45). The solid areas consist of sheets of spindled cells or closely packed solid or hollow tubules. The latter do not have a true luminal

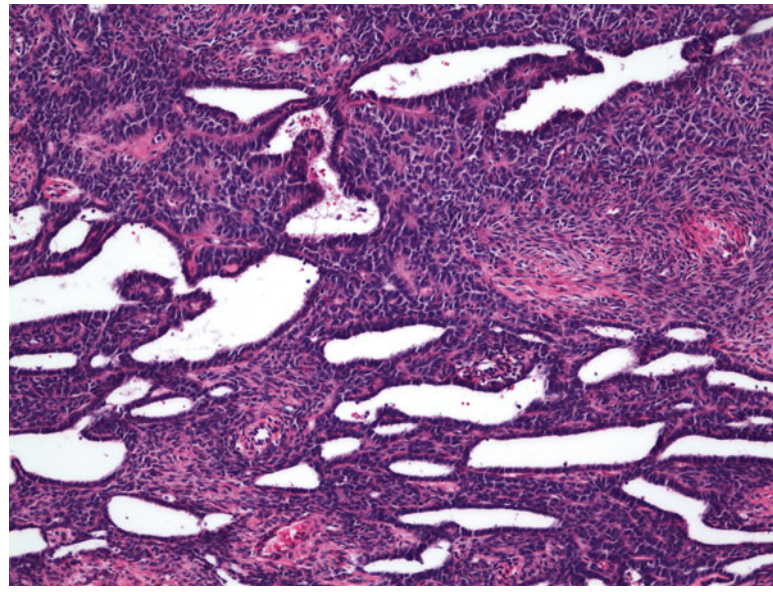

Fig. 17.43 Female adnexal tumor of probable wolffian origin. Cystically dilated tubules creating a sievelike appearance

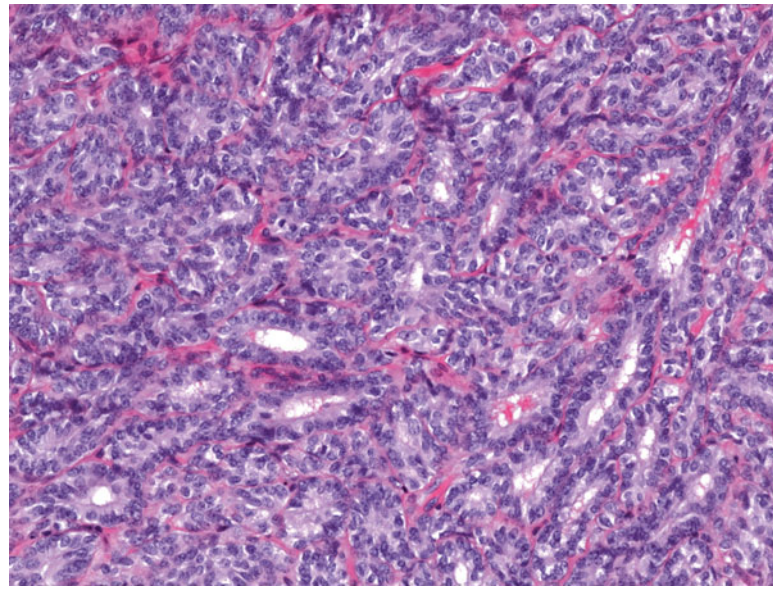

Fig. 17.44 Female adnexal tumor of probable wolffian origin. Closely packed tubules may mimic an endometrioid carcinoma

border. The cells are usually cytologically bland and mitotically inactive and have pale eosinophilic cytoplasm and oval and elongated nuclei. Some tumors have nuclear atypia and high mitotic rates and have been found to be clinically malignant. All FATWO should be considered a tumor of low malignant potential, as about 10 % were reported to be clinically malignant. They either have spread from their site of origin at the time of diagnosis or recurred years later. It should be noted that some of these clinically malignant tumors were cytologically bland. FATWO is usually immunoreactive for cytokeratins, estrogen and progesterone receptors, calretinin, CD10, and vimentin and focally for inhibin [150]. The most important differential diagnosis is from an endometrioid carcinoma, especially of tubal origin.

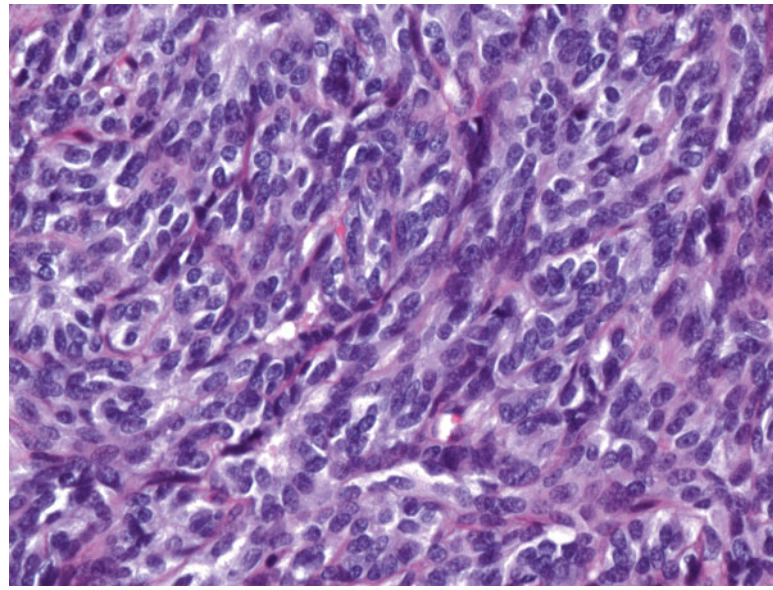

Fig. 17.45 Female adnexal tumor of probable wolffian origin. The tumor cells have bland cytology

Epithelial Tumors

Epithelial tumors of the broad ligament are usually of müllerian origin and include benign, borderline, and malignant types and are similar to those described for the ovaries. Some cases may be related to endometriosis, especially for clear cell carcinomas. The most common epithelial tumors are serous cystadenoma, followed by serous borderline tumor. The latter has been described in women 19–67 years of age (mean, 33 years) and usually presents as a mass. They are reported to be curative by surgery. Other benign or borderline epithelial tumors are less common.

A unique type of papillary cystadenoma involving the broad ligament has been described in patients with von Hippel-Lindau disease (VHL) [151–154]. These are probably of mesonephric origin. In some, the diagnosis of VHL is already known, while in others, this cystadenoma tumor was the initial manifestation of the disease. The tumors were usually cystic and contained complex papillae with fibrovascular stromal cores, covered by cuboidal nonciliated cells with bland nuclei. Some cases were bilateral.

Mesenchymal Tumors

Leiomyomas and leiomyosarcomas, respectively, represent the most common benign and malignant mesenchymal tumors of the broad ligament. Leiomyosarcomas are aggressive tumors and may affect both pre- and postmenopausal women. They are distinguished from those arising from the uterus or ovaries by their clear separation from these organs. The gross and microscopic features are similar to those of the uterine counterpart. Occasionally, they may contain osteoclast-like giant cells [155]. A case considered as tumor of low malignant potential was cured by surgery.

Other rarer benign and malignant mesenchymal tumors have been described, with lipoma being the more frequent type. Liposarcomas are exceptional.

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Pathology of the Peritoneum

18

Asma Zaman Faruqi

Abstract

This chapter gives an overview of the anatomy and histology of the peritoneum, nonneoplastic inflammatory and reactive peritoneal lesions, nonneoplastic and neoplastic Mullerian lesions, reactive and neoplastic lesions of the mesothelium, primary peritoneal carcinoma, pseudomyxoma peritonei, mucinous tumors of the peritoneum, primary mesenchymal tumors, metastatic disease, and benign lymph node inclusions.

Anatomy and Histology of the Peritoneum

The peritoneum is a serous membrane that invests most of the abdominal organs. It consists of two layers, the parietal and the visceral peritoneum. The parietal peritoneum lines the abdominal wall and the pelvic cavity, whereas the visceral peritoneum covers the abdominal organs. The peritoneal cavity is the potential space between these two layers; it contains about 50 ml of serous fluid which allows the two surfaces to slide smoothly over each other. In males the peritoneal cavity is closed, but in females there is communication with the exterior through the openings of the fallopian tubes; this anatomical feature increases the risk of peritoneal infection in females.

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The connective tissue present beneath the parietal peritoneum consists of fat, fibrous tissue and nerve bundles. The visceral peritoneum on the other hand is tightly attached to the abdominal and pelvic organs and separated from them by only a small amount of submesothelial fibrous connective tissue.

The cavity is divided into two parts: the greater sac and the lesser sac. The terms "intraperitoneal" and "retroperitoneal" are used to describe the relationship of various organs to the peritoneum. An intraperitoneal organ is almost completely covered by visceral peritoneum, whereas a retroperitoneal organ lies behind the peritoneum. The pancreas and ascending and descending colon are thus described as being retroperitoneal, whereas the stomach, jejunum, and ileum are regarded as intraperitoneal organs.

The peritoneal ligaments consist of double-layered folds of peritoneum that attach organs to the abdominal wall. In the female pelvis, the peritoneum from the anterior surface of the rectum reflects on to the upper posterior wall of vagina

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forming the rectouterine pouch (pouch of Douglas). It covers the posterior uterine surface, the fundus, the anterior surface of the uterus and reflects on to the superior surface of the urinary bladder and thence onto the anterior abdominal wall [1]. Due to the presence of the bladder, the peritoneal reflection on the anterior surface of the uterus is higher than that on the posterior surface, an arrangement which helps in the orientation of hysterectomy specimens.

Histology

Normal mesothelium consists of a single layer of small flattened cells with centrally located nuclei and a thin basal lamina which separates the mesothelial cells from submesothelial mesenchyme (Fig. 18.1a). Ultrastructurally, mesothelial cells have long slender microvilli. This feature gives rise to the characteristic "windows" seen between adjacent mesothelial cells in aspirates of serous fluids.

The submesothelial mesenchyme consists of collagen, elastic fibers, and blood vessels, including capillaries and lymphatics.

Immunohistochemistry: mesothelial cells express cytokeratins CK7 and CK5/CK6. In addition there is nuclear reactivity for WT1 and calretinin (Fig. 18.1b). D2-40, which recognizes human podoplanin, was initially used to delineate lymphatics, but it also marks mesothelial cells and the majority of epithelioid mesotheliomas (however, only about a quarter of sarcomatoid mesotheliomas are reactive). A subset of ovarian serous carcinomas may also be reactive for D2-40. The staining pattern is nuclear. Mesothelial cells are usually negative for BerEP4 and CEA. MOC 31 (also known as Ep-CAM or epithelial-specific antigen) is a useful addition to the panel. It recognizes glycoproteins on cell surface membrane and is positive in most epithelial cells with the exception of hepatocytes, squamous epithelium, gastric parietal cells, and proximal renal tubular cells.

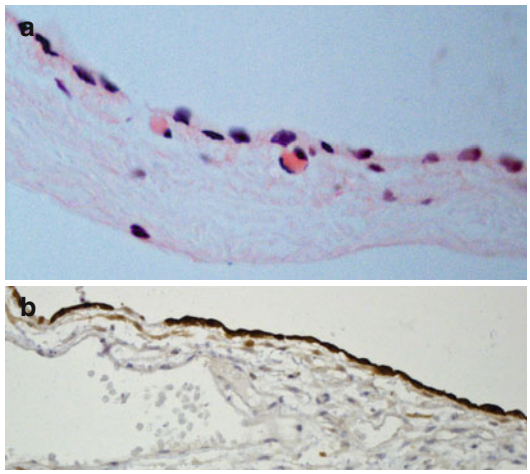

Fig. 18.1 (**a**) Normal mesothelium with flattened to cuboidal lining cells, (**b**) Normal mesothelium demonstrating nuclear reactivity for calretinin

Nonneoplastic Lesions

These include infectious, inflammatory, and reactive lesions.

Infections

Infectious peritonitis is usually bacterial in origin and often associated with rupture or infection of an intra-abdominal organ. It can therefore occur in appendicitis, diverticulitis, or pancreatitis. The pelvic peritoneum may also undergo inflammation due to ascending infection from the female genital tract (pelvic inflammatory disease).

Peritonitis results in exudation of fluid into the peritoneal cavity; the exudate is rich in neutrophils during the acute stage. When examined histologically, the peritoneal surface is covered by fibrinous material and there is infiltration by neutrophils, macrophages, and lymphocytes. Plasma cells may be present in long-standing inflammation. Clinically, the classical signs of tenderness (with rebound) and guarding are present. CA125 levels may be elevated and when there is an associated inflammatory mass, a clinical suspicion of an ovarian malignancy may arise.

Granulomatous Inflammation

Granulomatous inflammation may be of infectious or noninfectious etiology.

Tuberculous Peritonitis

According to estimates by the WHO, in 2010 there were about 8.8 million new cases of tuberculosis globally. Tuberculous peritonitis occurs when there is infection involving the intestinal tract, omentum, liver, spleen, or genital tract. It is relatively rare and accounts for 1–2 % of all cases of tuberculosis [2, 3]. Since pasteurization of milk became widespread in the West, abdominal tuberculosis has decreased; however, it still exists in large parts of Asia and Africa, especially in areas that have a high incidence of HIV/AIDS and multidrug-resistant disease. Tuberculous peritonitis may present as an abdominal mass, ascites, and elevated CA125 levels leading to a clinical suspicion of ovarian carcinoma. In most cases a histological diagnosis is required; the tissue may be obtained at surgery (laparoscopy or laparotomy) or by a radiologically guided biopsy of an omental mass. On histological examination classical tuberculous granulomata, comprising epithelioid cells and Langhans-type giant cells with or without caseous necrosis, are present (Fig. 18.2). Ziehl-Neelsen stain for demonstration of acid fast bacilli may be employed in order to confirm the diagnosis; however, a high bacterial load is required in order to detect acid fast bacilli with this method. The gold standard for diagnosis is therefore microbiological investigation. The methods used include culture and sensitivity, immunofluorescence and PCR. DNA for the latter technique may be extracted from formalin-fixed, paraffin-embedded tissue. Dispatch of material to microbiology by the surgeons is therefore advisable in cases of suspected tuberculosis.

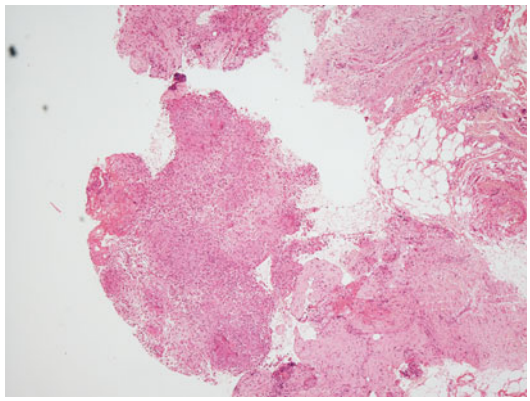

Fig. 18.2 Peritoneal tuberculosis. The patient was thought to have disseminated malignancy (Courtesy of Dr. Barry A.T. Newell)

Actinomyces

This may cause suppurative or granulomatous inflammation. Pelvic inflammation with actinomyces has been reported in association with intrauterine contraceptive devices (usually the copper coil) and vaginal pessaries. The typical bacterial colonies are yellow in color and described as "sulfur granules." On histology filamentous bacteria are identified which are positive with silver stains (Fig. 18.3a, b).

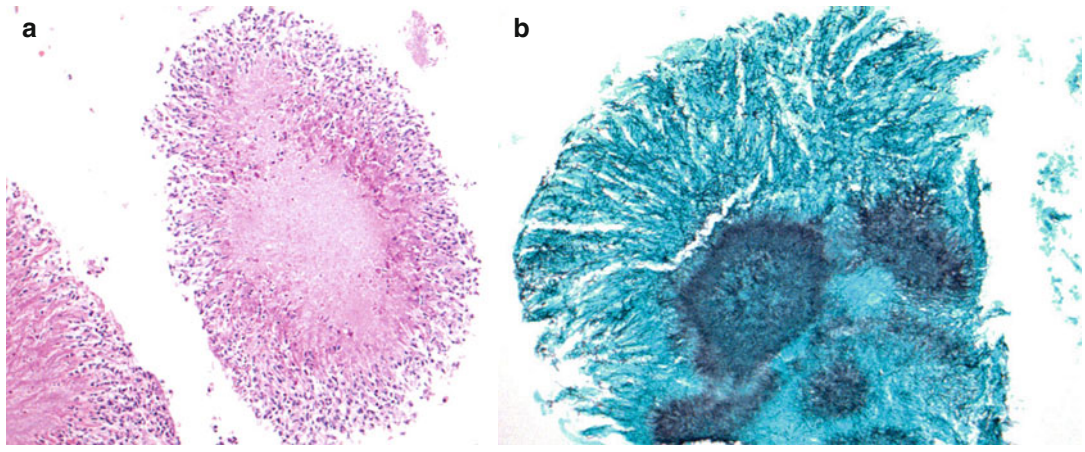

Fig. 18.3 (**a**) Actinomyces (H&E), (**b**) actinomyces with silver stain highlighting the filamentous bacteria

Noninfectious Granulomatous Inflammation

Sarcoidosis presents histologically with classical "naked" granulomata that consist of epithelioid cells and lymphocytes with no evidence of necrosis. Diagnosis of sarcoidosis requires full clinical assessment and estimation of serum ACE levels.

Foreign materials following surgical or radiological procedures may also lead to studding of the peritoneal cavity with granulomata. These may be due to suture material, talc, or radiological contrast media. Suture material or talc can be seen in foreign body-type giant cells; both are well visualized under polarized light, suture material as spicules and talc as "Maltese crosses" or rectangles (Fig. 18.4a, b).

Keratin granulomata can occur in ruptured cystic teratomas with aggregates of foreign body-type giant cells surrounding or containing eosinophilic, anuclear material. Keratin granulomata have also been reported in association with endometrioid endometrial carcinoma and ovarian carcinoma with squamous differentiation [4–7].

Certain hematological malignancies such as Hodgkin's lymphoma may elicit granulomatous inflammation and a biopsy taken from the edge of the lesion can be mistaken for an infectious granuloma or sarcoidosis (especially if there is lymphadenopathy) leading to a delay in diagnosis.

Reactive Changes

Mesothelial cells may undergo hyperplasia in response to a variety of irritative stimuli. Histologically, proliferation, tufting, and reactive atypia of the cells are observed (Fig. 18.5a, b). When the hyperplastic changes are florid, the condition needs to be distinguished from mesothelioma and primary peritoneal carcinoma. Although mitotic figures may occur, the mitotic index is generally low and there is an associated acute and chronic inflammatory infiltrate.

Lesions Associated with the Secondary Mullerian System/Mullerianosis

The primary Mullerian system comprises the paired Mullerian ducts that consist of the proximal free ends (the fallopian tubes) and the distal fused portion (the uterus, cervix, and upper vagina). Mullerian epithelium may therefore be tubal, endometrial, or endocervical in type.

The term secondary Mullerian system was used by Lauchlan in 1972 [8] and refers to the presence of Mullerian epithelium in sites other than the lining of the Mullerian ducts. He describes this, rather poetically, as the "shadow" cast by the primary Mullerian system. The secondary Mullerian system includes the mesothelium and

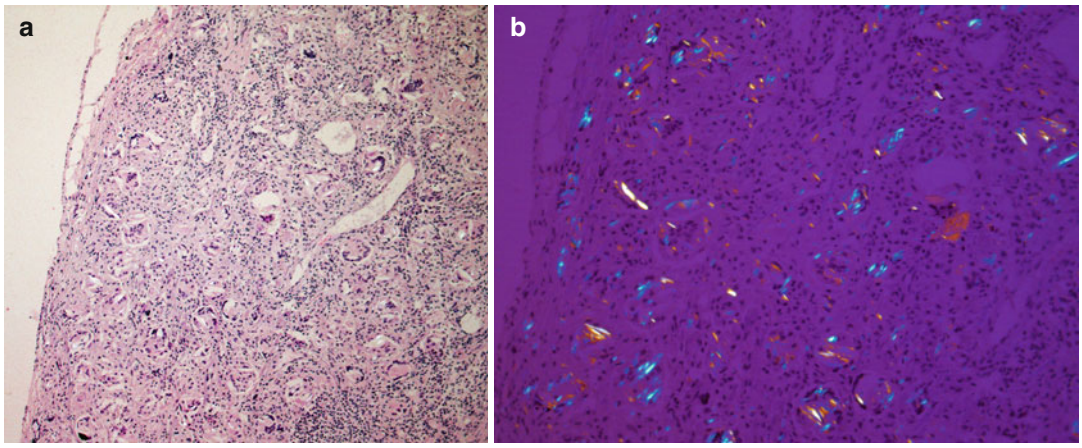

Fig. 18.4 (**a**) Foreign-body reaction following previous surgery. Giant cells with ingested foreign material. (**b**) Foreign-body reaction. Spicules of suture material seen under polarized light

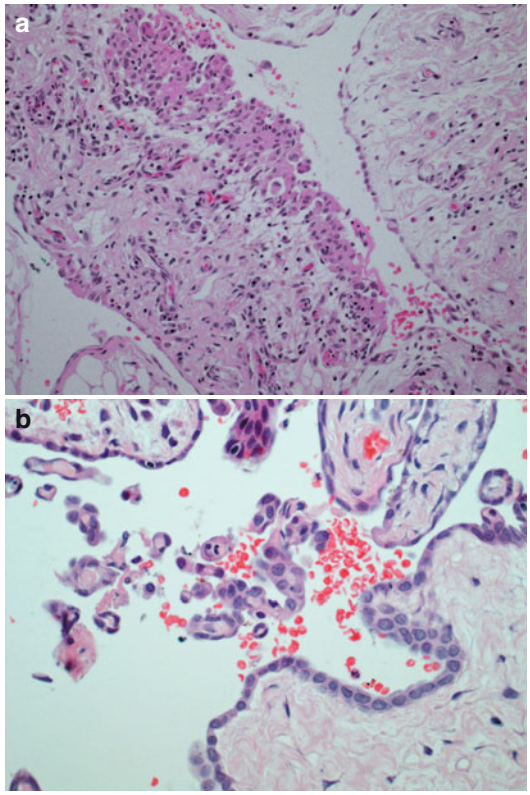

Fig. 18.5 (**a**) Mesothelial hyperplasia. Sheets of cells with inflammation of the submesothelial mesenchyme. (**b**) Mesothelial hyperplasia. Tufting of the cells and cytological atypia

the submesothelial mesenchymal tissue lining the pelvis and lower abdomen. Lesions of the secondary Mullerian system may contain a variety of Mullerian epithelia and many authors refer to them collectively as Mullerianosis. The etiology of these lesions is complex and has not been proved. It is thought that the submesothelial mesenchymal cell plays a role in their development; acting as a totipotential stem cell that can differentiate into various lineages [9]. It is suggested that at least some of the lesions may develop through a process of metaplasia or differentiation.

Endometriosis

Endometriosis is defined as the presence of endometrial glands and stroma at any location other than the lining of the uterus.

Incidence and Distribution

It is difficult to establish the incidence of this disease accurately; however, it is accepted that it occurs during the reproductive years and is usually discovered in the course of investigations of infertility or clinical symptoms such as pelvic pain, dysmenorrhea, and menorrhagia. An estimated 25–50 % of women with infertility and 5–50 % with pelvic pain are affected by endometriosis [10–12]. Nevertheless, many women with advanced endometriosis may have no symptoms; in one study a significant number of women undergoing tubal ligation were found to have hitherto unsuspected endometriosis [13]. The true incidence of this disease is difficult to investigate as it would require intrusive surgery on asymptomatic women which is obviously not feasible.

There have been various reports regarding the distribution of endometriosis, depending on whether the diagnosis was based on clinical findings or biopsy. On clinical examination, the two most frequent sites of endometriosis are the uterosacral ligaments and the ovaries; however, on biopsy the commonest sites are the ovaries, fallopian tubes, and uterine serosa. This discrepancy may be explained by the fact that in most cases endometriosis is diagnosed by history and clinical examination alone and certain sites such as the uterosacral ligament may not be biopsied [9, 14, 15]. Furthermore, in burnt-out disease, only fibrotic tissue may remain, and therefore, a definite diagnosis of endometriosis may not be possible.

Etiology and Pathogenesis

This is a subject of much research and debate with no single theory entirely explaining all the features of this disorder.

The implantation theory was proposed by Sampson [16, 17]. According to this endometriosis develops due to reflux of endometrial tissue through the fallopian tubes and implantation on the surfaces in the pelvic and lower abdominal peritoneum. It is proposed that this is the

mechanism by which endometriosis occurs in surgical scars and traumatized mucosa. In support of this theory are the observations that endometriosis is commonest around sites close to the fallopian tube, its association with tubal blockage, and more frequent occurrence in women with prolonged menstruation, heavy bleeding, and short cycles. Endometriosis in distant sites may be related to the passage of tissue via diaphragmatic defects which can account for deposits in the lungs and pleura.

Hematogenous spread to distant sites has also been postulated. Normal endometrial tissue has been demonstrated in endothelial lined spaces (Fig. 18.6) in the myometrium, and experimentally, tumor cells, dye, and radiographic material have been shown to migrate from the pelvis to the umbilicus by retrograde lymphatic dissemination [18, 19]. This could explain the presence of endometriosis in distant sites such as the lungs.

According to the theory of coelomic metaplasia, the mesothelial cells of the pelvic peritoneum can differentiate into Mullerian-type cells [8, 20]. This attempts to explain the presence of endometriosis in sites such as the pelvic lymph nodes. In support of this theory are the rare cases of endometriosis in subjects who would be unlikely to develop retrograde menstruation, such as women with Turner's syndrome or those with gonadal dysgenesis who are amenorrheic [21–23]. Endometriosis has also been reported in males [24–26], usually those receiving high-dose estrogens.

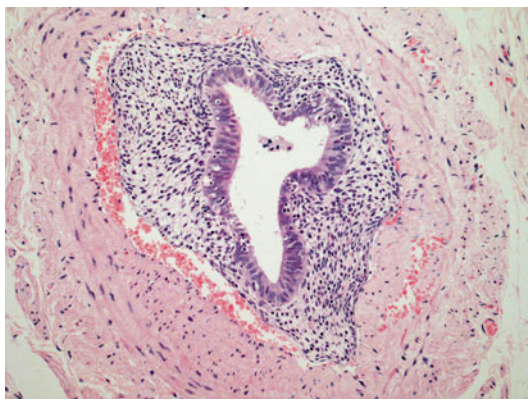

Fig. 18.6 A parametrial blood vessel containing endometrial tissue. The patient was menstruating

The induction theory postulates that endometrial cells themselves induce substances in the implanted tissue which support their growth. There is also a proposal that endometriosis arises from circulating stem cells in the blood which have the capacity to differentiate into endometriotic deposits at various sites [27].

It is now generally accepted that the main etiological factor in the development of endometriosis is retrograde menstruation leading to deposition of endometrial tissue in the pelvis. There have been numerous experiments demonstrating retrograde menstruation and this theory certainly seems logical and is supported scientifically. However, there must be additional risk factors that play a role; retrograde menstruation is a common phenomenon but endometriosis is apparently less widespread. It would seem that in women who develop endometriosis, there are mechanisms whereby the deposits are protected from local immune response and their growth and continued viability depend upon local factors, protection from apoptosis, and angiogenesis [28–30].

As endometriosis is a disease of reproductive life [31], the role of hormonal factors in its development and maintenance is logical. The rare reported examples of endometriosis in men and in women with gonadal dysgenesis generally occurred in association with hormonal therapy [21, 23, 32]. Unopposed or large-dose estrogens are implicated, whether through exogenous administration or peripheral aromatase conversion. Progestogens appear to have a protective function with a lower incidence among women who have used oral contraceptives or have had frequent pregnancies [33, 34].

There have been suggestions that familial or genetic factors may predispose to the development of endometriosis. However, this is controversial and yet to be proved. Molecular genetic analysis of endometriotic deposits has shown that these differ from eutopic endometrium from the same subject [35]. Researchers have demonstrated that endometriotic tissue contains increased cyclooxygenase-2 (COX-2) resulting in increased biosynthesis of prostaglandin E, estrogen, and cytokines such as interleukin 4 and interleukin 6 [36–38]. Compared to normal endometrium,

endometriotic lesions not only have fewer progesterone receptors but also appear to exhibit progesterone resistance [35]. SF1 is a transcription factor that mediates expression of steroidogenic genes in endometriotic stromal cells. SF1 is virtually absent in normal endometrium, but high levels of it are found in endometriotic tissue [39]; in addition estrogen receptor β (beta) and to a lesser extent α (alpha) are also considerably higher in endometriotic cells. Treatment of endometriosis has targeted aromatase with the use of inhibitors of the aromatase and COX-2 pathways.

Morphology

On laparoscopic examination endometrial deposits may take the form of white, red, or blue spots; dark brown, punctate foci ("powder burns") may also occur. Endometriotic cysts or endometriomas commonly occur in the ovaries, whereas complex, solid masses composed of endometriotic tissue, fat, and smooth muscle (nodular endometriosis) are most commonly seen in the rectovaginal septum. Endometriosis may also assume a polypoid form projecting into the lumina of cysts or the mucosa of the bowel.

Microscopically, classic endometrial implants consist of endometrial type glands surrounded by small stromal cells. Typical arterioles may be identified in the stroma (Fig. 18.7). The glandular epithelium may be inactive and proliferative or less frequently show secretory change.

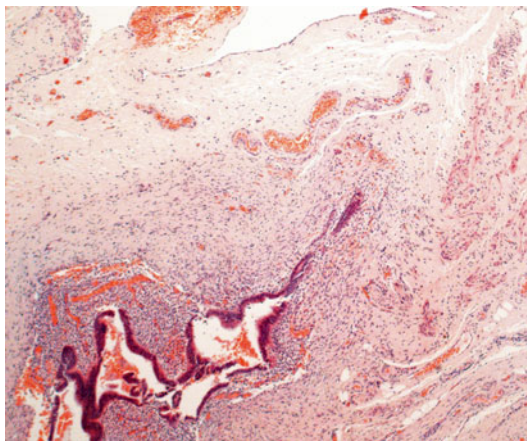

Fig. 18.7 Typical endometriotic focus. Endometrial glands surrounded by stroma containing blood vessels

The glands are often surrounded by fresh or old hemorrhage and histiocytes that contain pigmented material. The pigment may consist of hemosiderin in which case it appears as coarse, brown granules, but more commonly, it is ceroid (lipofuscin or hemofuscin) pigment which is fine and grey brown in color. Occasionally a massive histiocytic infiltrate can obscure the underlying stromal cells (xanthomatous endometriosis). In early lesions, the histiocytes may be nonpigmented with pigmentation occurring as the lesion ages.

The diagnosis of endometriosis may be more difficult when these typical lesions are altered by metaplastic or other changes [40].

Stromal Endometriosis

As the name indicates this consists of aggregates of stromal cells with no identifiable glands. The absence of glands may be due to sampling error, but it is also possible that some of the lesions are actually submesothelial mesenchymal cells that have undergone metaplastic changes. Stromal endometriosis is usually seen on the surface of biopsy specimens in the form of small nodules [41]. In some cases, the stromal cells may be inconspicuous and can be mistaken histologically for inflammatory cells (Fig. 18.8a). The presence of arterioles, telangiectatic vessels, and hemorrhage together with histiocytes should raise the suspicion that these are endometriotic deposits; this can be further confirmed by staining with CD10 and ER (Fig. 18.8b, c), which highlights endometrial stromal cells. The lesions should be differentiated from disseminated endometrial stromal sarcoma which is generally associated with a uterine lesion and lymphovascular invasion.

Metaplasia

Any of the metaplastic changes seen in normal endometrium may also be encountered in endometriotic deposits. These include ciliation,

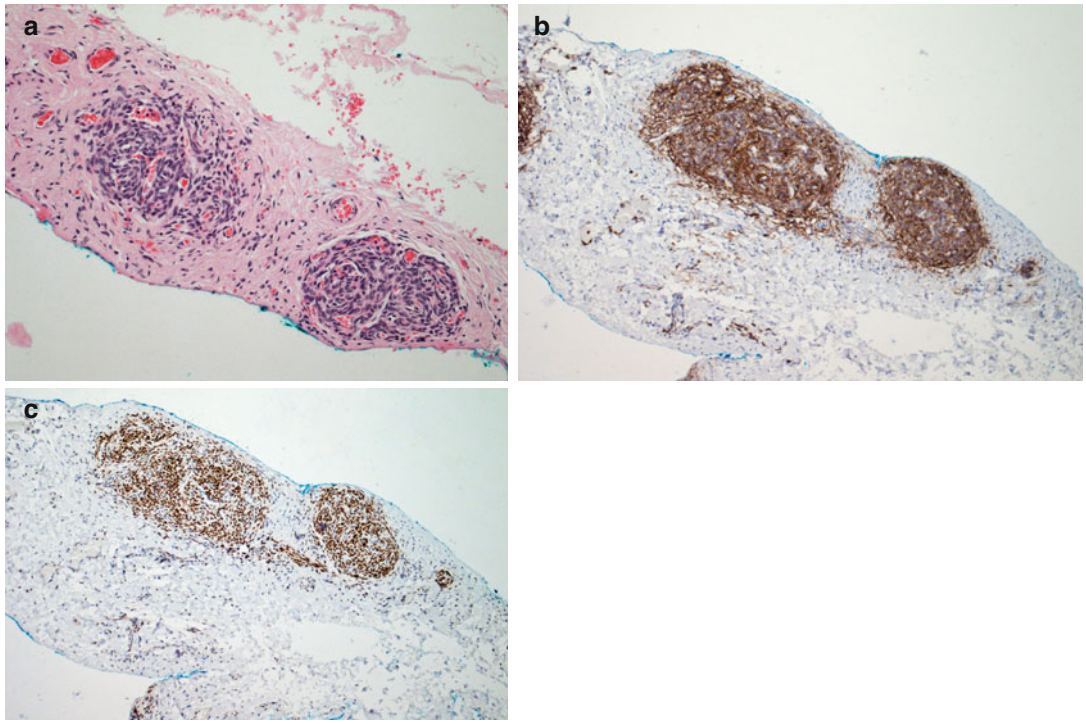

Fig. 18.8 (**a**) Stromal endometriosis (H&E), (**b**) stromal endometriosis showing reactivity for CD10, (**c**) stromal endometriosis demonstrating ER positivity

eosinophilic, mucinous, hobnail, and clear cell change. Mucinous metaplasia in ovarian endometriotic cysts may be associated with endocervical-type mucinous borderline tumors [42].

Smooth muscle metaplasia is typically found in ovarian endometriomas but may occur at other sites, such as the broad ligament and pelvic lymph nodes. In severe cases, it may give rise to uterus-like masses which need to be distinguished from Mullerian duct anomalies especially if associated with other congenital abnormalities [43–45].

Stromal myxoid change is rare; it results in deposition of myxoid material that is positive for stromal rather than epithelial mucin [40, 46]. In keeping with stromal mucin, the material stains with Alcian blue at pH 2.5 but is PAS negative.

Decidual alteration of the stroma is observed in pregnancy or treatment with progestogenic agents. The cells have abundant eosinophilic cytoplasm and round to ovoid nuclei (Fig. 18.9).

In some cases cytoplasmic vacuolation may occur which imparts a signet ring-type appearance to the cells. In such cases, the lesion needs to be differentiated from metastatic carcinoma [47]. Unlike metastatic carcinoma, decidual cells show an absence of the usual morphological features of malignancy and have bland nuclei with absence of reactivity for cytokeratin. In pregnancy the glands

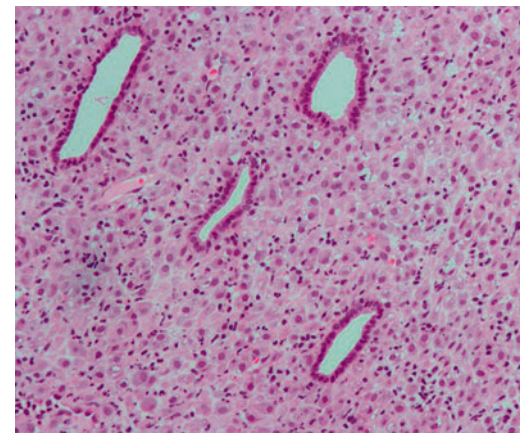

Fig. 18.9 Endometriosis with extensive decidual alteration of the stroma

may exhibit hypersecretory changes and Arias-Stella reaction. These may be mistaken for clear cell carcinoma; however, the clinical findings and the presence of decidualized stromal cells around the glands are useful in confirming endometriosis.

Atypical Endometriosis

Atypia may refer to cytological changes or architectural abnormalities (i.e., hyperplasia). Cytological atypia is common in endometriotic cysts and consists of cuboidal cells with enlarged nuclei, smudged chromatin, abundant cytoplasm, and occasional nucleoli. Hobnail cells and small papillae may also be identified. Generally, this is associated with a neutrophilic infiltrate and it is thought that these changes are reactive or degenerative if they occur as an isolated finding. In one study, a follow-up of 20 cases was uneventful with no reported malignancy [48]. Nevertheless, an association between severe cytological atypia and clear cell carcinoma or endocervical-type mucinous borderline tumors [40, 49, 50] has been reported. A study in which 36 endometriotic cysts were analyzed found aneuploidy in three of six cysts that had severe cytological atypia. In contrast, all the cysts with absent or mild atypia were diploid. The authors concluded that severe cytological atypia may be a precursor lesion for malignancy [51]. On a practical note, there are no definite guidelines regarding classification of these atypical changes into mild, moderate, or severe; therefore, considerable interobserver variability may occur. At the very least, follow-up would appear to be the prudent course of action.

Architectural atypia (hyperplasia) is much rarer than cytological atypia and therefore the significance of this finding is difficult to evaluate. It may occur with or without cytological atypia (Fig. 18.10). As there is a reported association with carcinoma [52–54], extensive sampling is recommended if this feature is encountered. As before follow-up is advisable.

Polypoid endometriosis refers to endometriosis resembling an endometrial polyp [55]. It can occur at a variety of sites and may present as a complex pelvic mass or large bowel obstruction

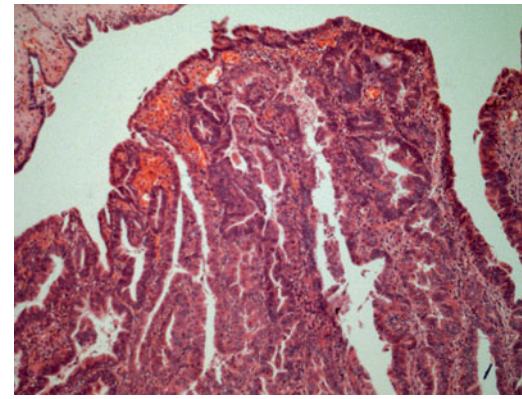

Fig. 18.10 Atypical endometriosis. There is architectural atypia with crowding and complexity of the glands

from a mucosal or mesenteric lesion [56, 57]. Unlike usual endometriosis, polypoid endometriosis is often seen in postmenopausal women. The lesions are composed of variable-sized glands which can exhibit a variety of metaplastic changes. Usually there is no significant periglandular stromal condensation and stromal atypia is lacking, features that differentiate the lesions from extrauterine Mullerian adenosarcomas.

Necrotic Pseudoxanthomatous Nodules (NPN)

These may occur at single or multiple sites; they consist of a necrotic core surrounded by histiocytes often in a palisaded arrangement [58] (Fig. 18.11). Endometrial glands or stroma are often inconspicuous and it is likely that the lesion represents end-stage endometriosis. One case report describes disseminated nodules following a ruptured endometriotic cyst [59]. When present at multiple sites, the clinical impression may be of disseminated malignancy, and if the central necrotic area is sampled at frozen section, it may give rise to further concern.

Liesegang Rings

These are zones of precipitation from colloid-containing supersaturated solutions [60]. They are variable-sized, acellular eosinophilic ring-like

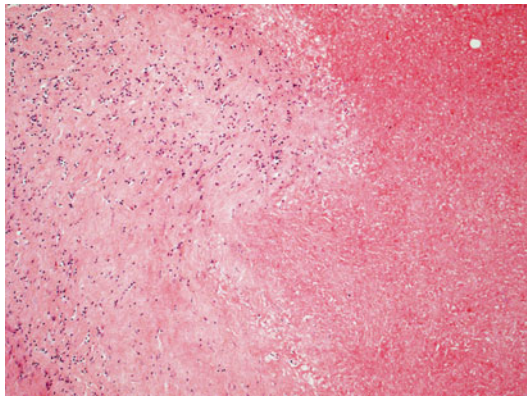

Fig. 18.11 Necrotic pseudoxanthomatous nodule

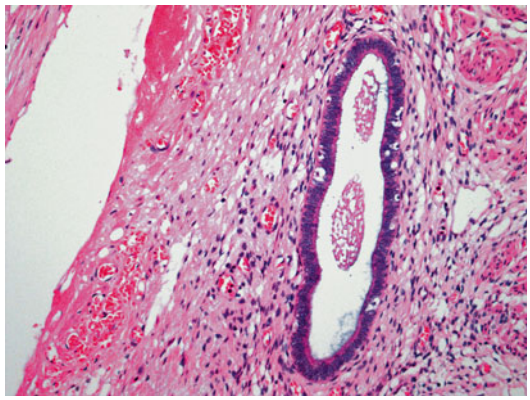

Fig. 18.12 Peritoneal endosalpingiosis

structures that are often found adjacent to foci of hemorrhage and necrosis. In the female genital tract, there is an association with endometriosis [61–63]. Histologically they may be mistaken for psammoma bodies (due to lamination) or parasitic larvae or worms.

Endosalpingiosis

This is the presence of benign glands lined by fallopian tube-type epithelium which may be associated with psammomatous calcification. It occurs in the peritoneum, retroperitoneum, or pelvic lymph nodes and is usually an incidental finding. The glands are variable in size and lined by a single layer of typical, ciliated, and nonciliated epithelial cells with intercalated peg cell and basal lymphocytes (Fig. 18.12). The cells express WT1 in keeping with their serous origin. The surrounding stroma is composed of loose connective tissue.

Atypical endosalpingiosis: this is a term used to describe foci of endosalpingiosis that show tufting, stratification, cribriform architecture, or cytological atypia in circumstances where there is no serous borderline tumor present [64].

Endocervicosis and Other Mucinous Lesions

The presence of mucinous epithelium has been documented on the ovarian surface [8, 20] and at other sites, most commonly the urinary bladder [65–67] but also the outer cervix, paracervical tissue [68], vagina [69], pelvic lymph nodes [70], retroperitoneum [8], and intestine [71]. The term endocervicosis is used because the epithelial cells have a picket fence arrangement with apical, dispersed mucin and are thus morphologically similar to endocervical cells. Ciliation is sometimes observed supporting the Mullerian origin of these cells. The epithelial cells may occasionally exhibit mild atypia but are not generally mitotically active; it is important to bear this in mind, as mildly atypical glandular lesions at the above sites are not necessarily adenocarcinoma.

Mucinous tumors may occur at extraovarian sites and show the full range of epithelial abnormalities, benign, borderline, and malignant [72–74]. A recent report documented malignant transformation of presumed extraovarian mucinous cystadenoma that presented as a retroperitoneal mass and did not appear to have arisen in either the adjacent ovary or the colon [74]. The lesion showed a range of epithelial abnormalities from benign-looking glands through to epithelium with cytological and architectural atypia; metastatic deposits were present in the regional lymph nodes. Molecular analysis showed KRAS mutations in the atypical epithelium but not in the benign-looking glands.

Sheet-like histiocytic proliferations in the pelvic peritoneum have also been reported [75]. These were found in association with mucinous glands resembling endocervical glands.

Deciduosis

This refers to the presence of decidua at ectopic sites. It is usually seen in the pelvic peritoneum in pregnancy; less commonly, pelvic lymph nodes, subcapsular ovarian stroma, and the fimbrial end of the fallopian tube [9, 76–80] may be involved. Rare cases have been reported in women taking exogenous hormones, those with trophoblastic disease, or those who have undergone pelvic irradiation [9, 81]. Some of the cases may be endometriotic deposits that have undergone extensive decidual alteration and a search should be made for residual endometrial glands. Nevertheless, not all cases are due to endometriosis and like stromal endometriosis; focal deciduosis may also originate in the submesothelial stromal cells. The lesions consist of sheets of cells with abundant, eosinophilic cytoplasm, round to ovoid nuclei, and well-defined cell borders. Deciduosis may be a cause of extensive intraperitoneal postpartum hemorrhage.

Ectopic decidua should be differentiated from deciduoid mesothelioma, especially if the cells exhibit cytological atypia. Mesothelial cells are positive for broad-spectrum cytokeratins and calretinin, which is not the case with decidual cells.

Transitional Epithelium

Brenner tumors and related neoplasms may develop from transitional epithelium. Epithelial rests (Walthard cell nests) are usually located in the soft tissue around the fallopian tubes and ovaries (Fig. 18.13). These rests may give rise to benign or malignant transitional type tumors.

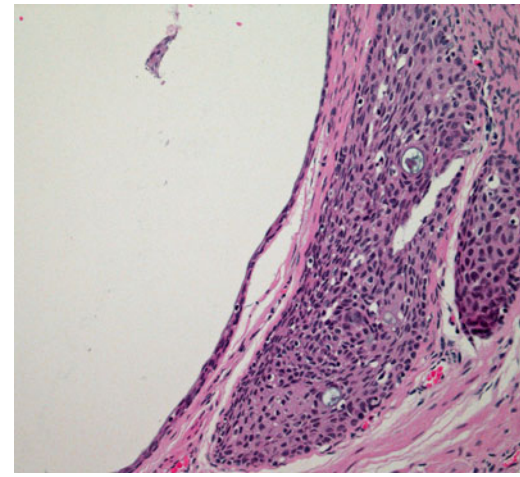

Fig. 18.13 Walthard cell nest. A cyst with attenuated transitional lining and islands of similar cells in the subjacent tissue

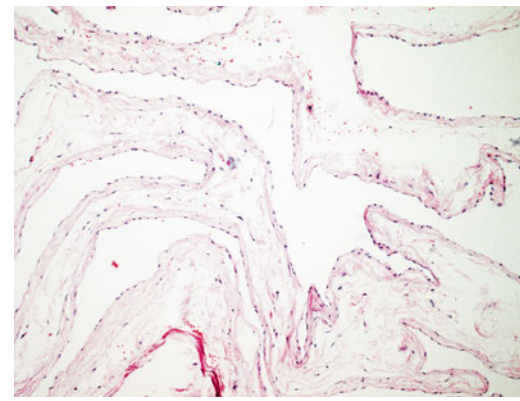

Fig. 18.14 Benign mesothelial cyst

Mesothelial Lesions

Unilocular Mesothelial Cyst

These are simple cysts lined by bland mesothelial cells that are thought to be inclusion cysts or reactive lesions rather than neoplasms (Fig. 18.14). They are usually found in the peritoneal cavity in women and may be single, multiple or even free-floating [82].

Multicystic Mesothelial Proliferation (Benign Multicystic Mesothelioma, Multilocular Peritoneal Inclusion Cyst)

There is debate in the literature about whether this lesion is neoplastic or reactive. Some authors consider it an indolent neoplasm, whereas others feel it is hyperplastic in nature [82–89]. It usually occurs in premenopausal women; common sites include the pouch of Douglas, the surface of the uterus or rectum and very rarely the ovary [90, 91]. Rare cases in men have also been reported, generally in the spermatic cord, pleura, or pericardium [92–96].

There is an association with disorders that can cause inflammation and fibrous adhesions, such as endometriosis, previous surgery, chronic inflammation, and diverticulitis [40, 83, 84, 94, 97–99]. This is considered as confirmation of the reactive nature of this lesion by the proponents of the nonneoplastic theory; others feel that as some cases have no history of inflammation, they are likely to represent neoplasia. Reports of recurrences and a transition to malignant mesothelioma would appear to give some support to this latter theory. Currently, there is a tendency towards more aggressive management of these lesions.

The lesion consists of multiple, variable-sized cysts lined by cuboidal or flattened cells; the subjacent stroma may be fibrotic or inflamed, and in some cases foci of endometriosis may be observed in the fibrous septa or in adjacent tissue. Adenomatoid foci, squamous metaplasia, clear cell change, and occasional hobnail cells have been described.

Neoplastic Lesions

Primary Mesothelial Neoplasms

Adenomatoid Tumor

This is a benign mesothelial neoplasm. The usual sites of involvement in women are the fallopian tubes, uterus, and ovary. However, there have been occasional reports of adenomatoid tumors in the omentum and liver [100–102].

It is usually an incidental finding and consists of tubules or sheets of cuboidal or flattened cells. The immunoprofile confirms the mesothelial origin of the cells (positive for calretinin, WT1, D2-40, and broad-spectrum cytokeratin; negative for vascular markers such as CD31 and epithelial markers BerEp4 and MOC31).

Multicystic Mesothelial Proliferation

See discussion above.

Well-Differentiated Papillary Mesothelioma (WDPM)

This is an indolent or borderline tumor, which usually occurs in the peritoneal cavity and is much commoner in women although rare cases have been described in men [103–105]. The localized form of the tumor generally has no association with asbestos exposure [82]; however, in one series of fourteen cases, an identifiable history of asbestos exposure was found in six [77]. The tumor is typically an incidental finding; when multifocal it may give an impression of peritoneal carcinomatosis. A recent article reviewed the features in 26 females with WDPM [106]. Only two of the patients presented with symptoms, the remaining were incidental diagnoses. There was no history of asbestos exposure in 25 of the cases (clinical history was unavailable for one), 10 patients had a history of previous surgery, and 6 had associated endometriosis. The lesions were single as well as multiple and varied in size from 10 to 20 mm; the majority was nodular or papillary in appearance. Recurrence, discovered incidentally during unrelated subsequent surgery, occurred in one patient; none of the patients died of the disease. The authors conclude that this should be regarded as a neoplasm of uncertain malignant potential requiring follow-up.

According to the criteria for diagnosis, tumor should be confined to the peritoneal surface with no invasion into the underlying stroma. The neoplasm has a characteristic papillary architecture, the papillae being covered by bland, cuboidal cells with fibrovascular cores, which may undergo myxoid change. Adenomatoid foci, cystic areas, multicystic mesothelial proliferation, squamous metaplasia, clear cell change, and occasional hobnail cells have all been described. Solid foci, psammomatous calcification, or ossification [107] can sometimes be seen. Cytologically, the cells are bland with no evidence of necrosis or mitoses. Immunohistochemistry reveals reactivity for calretinin and D2-40.

The differential diagnosis includes benign, reactive mesothelial proliferation, malignant mesothelioma, and borderline serous tumor. Reactive mesothelial proliferation may exhibit a papillary architecture, but this usually occurs as tufts or buds without fibrovascular cores and there is an inflammatory infiltrate or fibrosis [106]. Malignant mesothelioma should be excluded by clinical and radiological correlation and extensive sampling. Invasion of the underlying stroma should be

assiduously looked for; bulky disease, cytological atypia, mitotic activity, solid sheets of tumor cells, and a markedly complex papillary pattern are features of malignant mesothelioma rather than WDPM. Borderline serous tumors may bear a morphological resemblance to WDPM, but on immunohistochemistry, these are negative for calretinin and D2-40 and positive for BerEp4 and MOC31.

Malignant Mesothelioma

Diffuse malignant mesothelioma of serous membranes is a neoplasm that grows on serosal surfaces and does not arise from an underlying organ. It may present as small nodules, plaque-like sheets, or a tumor mass and is far more common in the pleura than the peritoneum. As it is commoner in males, most literature has concentrated on this aspect. The ratio of pleural to peritoneal neoplasms in men is 5:1; in women, peritoneal mesothelioma is relatively more common, the ratio being 2:1 [108–112].

As this is an uncommon neoplasm, it can often be misdiagnosed as serous carcinoma of ovarian, tubal, or primary peritoneal type. Unlike pleural tumors, peritoneal mesothelioma in women does not usually have a link to asbestos exposure [113, 114]. Occasional cases have been described following radiotherapy [109, 115–117]. There have been reports of pleural mesothelioma associated with SV-40 virus exposure (due to the contamination of polio vaccine with this virus) [118–121]. Although the virus is known to cause disease in animals and SV-40 sequences have been isolated in pleural tumors, viral etiology for the development of human mesothelioma is disputed [122, 123]. Currently, no cases of peritoneal mesothelioma have been reported in association with SV-40 virus. A group of villages in central Turkey have a high incidence of mesothelioma which is due to an asbestos-sized fiber found in volcanic rock and soil.

Mesotheliomas may be divided histologically into epithelial (epithelioid), sarcomatous (sarcomatoid), desmoplastic (variant of sarcomatoid), and mixed (biphasic) types. It is important to separate epithelial from sarcomatoid tumors because this is of prognostic significance; sarcomatoid mesotheliomas are resistant to treatment by radiotherapy or chemotherapy [124]. The subclassification of various types of epithelioid mesothelioma does not confer any prognostic significance but is a histological classification, which aids recognition.

Epithelioid Malignant Mesothelioma

This may have a sheet-like, glandular, or papillary architecture, the cells appearing rather monomorphic and bland. They can be cuboidal, polygonal, or flattened and the cytoplasm is eosinophilic and often dense (Fig. 18.15a, b). Mitotic figures may be inconspicuous.

Deciduoid mesothelioma is a rare variant that consists of large polygonal cells with abundant, eosinophilic cytoplasm imparting a superficial resemblance to decidua [125–127]. These lesions have been described in the peritoneal cavity in women but have also been reported in the pleura and in men [125].

Epithelioid mesothelioma may sometimes present as a markedly pleomorphic tumor which makes differentiation from metastatic carcinoma difficult on morphology alone.

Other variants of epithelioid mesothelioma include small cell mesothelioma and tumors associated with clear cells. Small cell mesotheliomas are positive for broad-spectrum cytokeratins and usually stain for NSE but not chromogranin [128]. Unlike small cell carcinoma, the mitotic index is generally low.

Epithelioid mesotheliomas may generate a marked desmoplastic response leading to trapping of the neoplastic cells within fibrous tissue that can resemble metastatic breast carcinoma (Fig. 18.15c). Sometimes mucin-like cytoplasmic droplets or even signet ring-type cells may be seen; these are negative for stains that highlight neutral mucin such as PAS after diastase digestion.

Sarcomatoid Mesotheliomas

These tumors may have a variety of appearances but often consist of spindle cells which possess a storiform or fascicular appearance (Fig. 18.16a). The cell morphology may be bland but is more often atypical, with pleomorphic cells, including giant cells. Heterologous elements such as bone and cartilage can also be seen. The differential diagnosis includes sarcomatoid carcinoma, malignant fibrous histiocytoma, and other soft

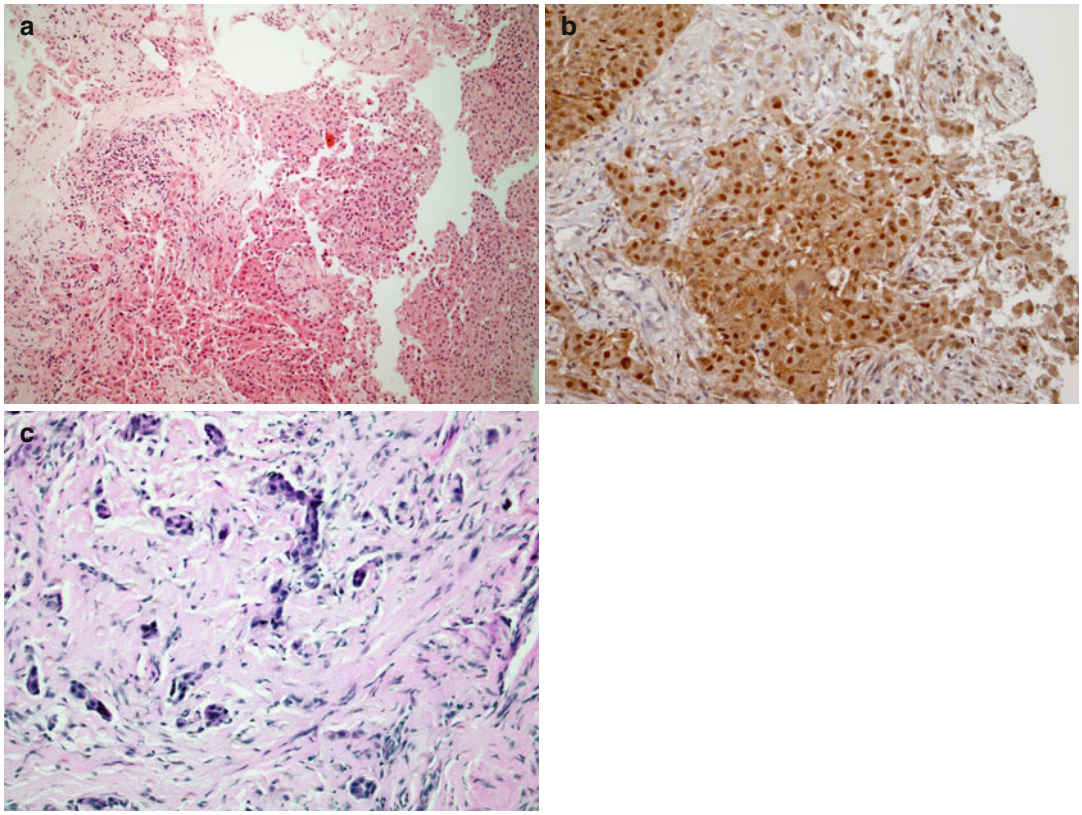

Fig. 18.15 (**a**) Epithelioid mesothelioma. Rather monomorphic cells with dense eosinophilic cytoplasm, (**b**) epithelioid mesothelioma exhibiting nuclear reactivity for calretinin. (**c**) Epithelioid mesothelioma – there is a resemblance to metastatic breast carcinoma

tissue sarcomas such as leiomyosarcoma, fibrosarcoma, and synovial sarcoma. Most sarcomatoid mesotheliomas express broad-spectrum cytokeratin, although only focal staining may be encountered; rare tumors are negative for cytokeratin [82].

Variants of sarcomatoid mesothelioma include desmoplastic and the extremely rare lymphohistiocytic mesothelioma. Desmoplastic malignant mesothelioma is a variant of sarcomatoid mesothelioma that is composed of densely collagenized tissue with neoplastic cells often in a storiform pattern (Fig. 18.16b, c). The tumor is paucicellular and can be mistaken for reactive fibrosis. Tumor cells infiltrating into the underlying tissue, necrosis, and overtly sarcomatous foci are required for diagnosis.

Sarcomatoid mesotheliomas may express broad-spectrum cytokeratins, SMA, desmin, S100, and CD68 [129]. Clinicopathological correlation and a panel of immunohistochemical stains are recommended in order to differentiate the neoplasm from soft tissue sarcoma. Positivity for Cam5.2, WT1, AE1/3, calretinin, and D2-40 is suggestive of sarcomatoid mesothelioma. In difficult cases recourse to molecular and ultrastructural studies may be required.

Biphasic tumors consist of mixed epithelial and sarcomatoid components. The transition from the epithelial to the spindle component may be gradual or abrupt.

A large review studied 75 cases of peritoneal malignant mesothelioma in females [109] in which the authors highlighted the morphological features. The age range in this series was 17–92 years and the clinical presentation was usually with abdominal or pelvic pain, abdominal swelling, ascites, or a pelvic mass. The majority of cases had widespread disease at the time of laparotomy; in a minority, disease was limited to the bowel or omentum. Seventy of the tumors had an epithelial morphology, four were biphasic and

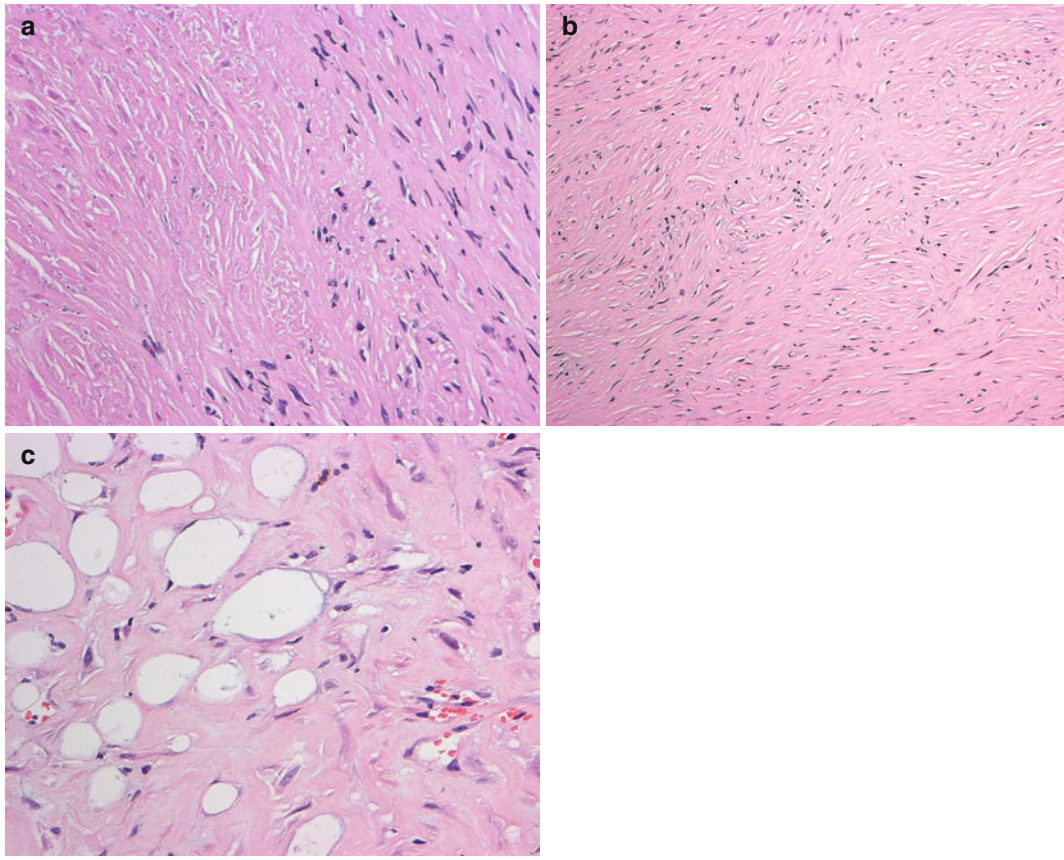

Fig. 18.16 (**a**) Sarcomatoid mesothelioma. Tumor necrosis in the left field; note the shadows of enlarged atypical nuclei. Better preserved spindle cells with subtle atypia on the right (Courtesy of Prof. M.T. Sheaff). (**b**) Desmoplastic mesothelioma. Spindle cells in a collagenized stroma. The appearances can easily be mistaken for fibrosis (Courtesy of Prof. M.T. Sheaff). (**c**) Desmoplastic mesothelioma. Infiltration of underlying adipose tissue, nuclear atypia is minimal (Courtesy of Prof. M.T. Sheaff)

one was purely sarcomatoid. The commonest epithelial patterns identified were tubular, papillary (often with hyalinized cores), and solid. Some cases exhibited slit-like glandular lumina reminiscent of serous carcinoma. In one case there was a strong resemblance to a sex cord stromal tumor with the presence of nuclear grooves, solid nests, trabeculae, and sertoliform tubules. About 20 % of cases, predominantly those with a papillary architecture, were associated with psammoma bodies. The degree of nuclear atypia ranged from mild (31 cases) to moderate and severe (10 cases). The single sarcomatoid mesothelioma in the series had a fascicular and storiform pattern with foci of abundant collagen, necrosis, severe cytological atypia, and frequent mitoses. There was limited invasion of the intra-abdominal organs.

Differential Diagnosis

Mesotheliomas usually have cuboidal cells with eosinophilic cytoplasm and mild to moderate nuclear atypia, thus resembling reactive mesothelial cells. Tufting and budding are less likely and tubules are small and evenly spaced. When there is a papillary architecture, papillae are orderly and well developed, covered by a single layer of mesothelial cells, and show hyalinized cores. Although psammoma bodies and slit-like spaces may be present, they are generally rare. These features should differentiate malignant mesothelioma from papillary serous carcinoma. Clear cell carcinoma of the female genital tract may have hyalinized cores, but the cells exhibit a marked degree of nuclear atypia often accompanied by hobnail cells and occasional intracytoplasmic eosinophilic globules. In contrast, when

cytoplasmic clearing is seen in malignant mesotheliomas, it is generally focal and more typical features of mesothelioma can be identified in other areas. The heterogeneity in the morphology of malignant mesothelioma should be emphasized particularly in regard to biopsy material. This is especially the case when the differential diagnosis lies between malignant mesothelioma and well-differentiated papillary mesothelioma, a tumor that is indolent and has a much better prognosis. In order to reliably distinguish these two tumors, it is essential to remove the entire lesion for histological assessment.

A panel of immunohistochemical stains is essential to differentiate mesothelioma from primary or metastatic carcinoma (Table 18.1).

Table 18.1 Differential diagnosis – mesothelioma, serous carcinoma, female genital tract clear cell carcinoma

	Mesothelioma	Serous Carcinoma	Clear Cell Carcinoma
BerEp4	Negative	Positive	Positive
CD15	Negative	Positive	Positive
MOC31	Negative	Positive	Positive
B 72.3	Negative	Positive	Positive
CK5/6	Positive	Negative	Negative
Thrombomodulin	Positive	Negative	Negative
Calretinin	Positive	Negative	Negative
D2-40	Positive	Rare	Negative
WT1	Positive	Positive	Usually negative
ER	Usually negative	Positive	Usually negative
HNF 1beta	Negative	Negative	Positive
Hyalinised papillary cores	Positive	Focal	Often positive
Hobnail cell and detached clusters	Usually negative	Positive	Positive
Nuclear atypia	Usually mild to moderate	Often severe	Often severe
Slit-like spaces	Occasional	Typical	Infrequent
Eosinophilic cytoplasmic globules	Uncommon	Uncommon	Typical
Dense eosinophilic cytoplasm	Typical	Uncommon	Uncommon

Adapted from Baker et al. [109]

Primary Peritoneal Carcinoma

This will be dealt with briefly in this section; a more detailed discussion will be found in the chapters on the ovary and fallopian tube.

The criteria for the diagnosis of primary peritoneal carcinoma according to the Gynecologic Oncology Group [130] are:

1. Both ovaries must be normal sized or enlarged by a benign process.
2. Extraovarian involvement must be greater than ovarian involvement.
3. The ovarian component must be either nonexistent or confined to the surface with little or no cortical invasion, the size not exceeding 5×5 mm.
4. The tumor must be of serous cell type.

Primary peritoneal carcinoma may be borderline, low grade, or high grade.

Borderline Serous Tumors

These usually occur in young women of reproductive age and present as widespread miliary involvement of the peritoneum and omentum. Sometimes the disease may be focal and only discovered incidentally at laparotomy. The neoplasms resemble ovarian borderline tumors and consist of a papillary surface proliferation of epithelial cells with low-grade nuclei and few mitotic figures [64, 131]. An association with endosalpingiosis is reported in 85 % of cases. The prognosis is favorable, although very rarely transformation to a low-grade papillary serous carcinoma may occur.

Low-Grade Papillary Serous Carcinoma

Morphologically these resemble borderline serous tumors but exhibit stromal invasion.

Psammocarcinoma

This is a variant of low-grade papillary serous carcinoma. It is defined as a tumor in which at least 75 % of the papillae are associated with psammoma bodies. Solid epithelial proliferation should not be present [132–134].

High-Grade Serous Carcinoma

Most primary peritoneal carcinomas are high grade and comprise cells with pleomorphic nuclei, normal and abnormal mitotic figures, papillary tufting, and detached cell clusters.

The etiology and pathogenesis of primary peritoneal carcinoma is the subject of much debate. Due to the high mortality rate of ovarian carcinoma, pathology specimens are now being examined in greater detail and cases classified as primary peritoneal carcinoma have risen from 10 % to about 18–28 % [135–137]. If it is accepted that the pelvic peritoneum is part of the secondary Mullerian system, then primary peritoneal carcinoma could arise through a process of metaplasia or due to malignant transformation of endosalpingiosis. On the other hand, there are now numerous detailed studies following examination of the tubal fimbriae in women with serous pelvic carcinoma [138–146]. The fallopian tube was first postulated as the site of origin of serous carcinoma by Alban Doran in 1896 [147]. This concept was intermittently revisited until Piek et al. [148] published a paper describing fallopian tube dysplasia in women with a genetic predisposition to ovarian cancer. It has now been accepted that a significant number of cases of primary peritoneal carcinoma are associated with serous tubal intraepithelial carcinoma (STIC). It is postulated that tumor cells from the fallopian tube implant on the surface of the peritoneum and the ovary giving rise to serous pelvic carcinoma. Both theories have their proponents and it is possible that the truth lies somewhere in between [149].

The etiology of the lesions is multifactorial. The evidence that some women have a familial predisposition to developing pelvic carcinoma is incontrovertible. Other factors that have been implicated include pelvic inflammatory disease and inflammatory agents associated with retrograde menstruation (interleukin 8, interleukin 12, granulocyte-macrophage colony-stimulating

factor, and tumor necrosis factor alpha). It has been suggested that talcum powder may play a role in the development of serous carcinoma. Talcum powder (magnesium trisilicate in its pure form) may contain a mixture of minerals, including asbestos-like fibers such as tremolite, anthophyllite, and chrysotile [150–153], and particles of talcum powder have been found deeply embedded within ovarian tumors [151]. The male peritoneum is a closed cavity; in contrast, the female peritoneal cavity is open and therefore vulnerable to dusting of the genital area with talcum powder. There appears to be a reduced risk of pelvic serous carcinoma with increasing parity [154–156], use of oral contraceptives for a period of 5 years or longer [157, 158] and after tubal ligation and hysterectomy [159–161].

Peritoneal Implants

These are associated with borderline serous tumors of the ovary and will be discussed in detail in the relevant chapter.

Mucinous Ascites and Pseudomyxoma Peritonei (PMP)

Pseudomyxoma peritonei is a clinicopathological syndrome [162] in which a mucinous neoplasm is associated with mucinous ascites ("jelly belly"). Pools of extracellular mucin and fibrosis are present in the peritoneal cavity and there is a characteristic scalloped appearance on imaging (Fig. 18.17a, b).

Peritoneal biopsies reveal pools of mucin containing strips of epithelial cells. The appearances may be bland, resembling a mucinous adenoma (a condition referred to as disseminated peritoneal adenomatosis, DPAM), or the cells may have moderate to marked atypia (peritoneal mucinous carcinomatosis, PMCA) (Fig. 18.17c). It is now generally accepted that pseudomyxoma peritonei is due to a primary gastrointestinal neoplasm and that ovarian involvement is secondary [163–166]. Rupture of primary ovarian mucinous tumors has not been associated with this condition [167]. The neoplasm is usually in the appendix although other sites in the gastrointestinal tract may be involved. The appendix may not necessarily appear abnormal and the tumor may only be found on microscopic examination. If a borderline or malignant mucinous ovarian neoplasm is diagnosed at intraoperative frozen section, the gastrointestinal tract should be thoroughly explored and the appendix should be removed and examined in its entirety. Very rarely pseudomyxoma peritonei may be associated with a gastrointestinal tumor arising in a mature cystic teratoma [168, 169].

Extraovarian Mucinous Neoplasms

Rarely, ovarian-type mucinous neoplasms may occur in the peritoneum, or (more commonly) the retroperitoneum, in the absence of an ovarian tumor. Some of the tumors may arise in a supernumerary ovary. However, in most cases although ovarian-type stroma may be identified, follicles or sex cord like structures are absent; furthermore, as they can also, albeit rarely, occur in males, it is quite possible that they are peritoneal in origin. The histological appearances encompass the whole spectrum from benign mucinous cystadenoma through tumors of borderline malignancy to frank mucinous carcinoma [72, 73, 170, 171]. Some tumors contain mural nodules with features of anaplastic carcinoma, similar to those seen in ovarian mucinous neoplasms.

Carcinosarcoma

Extrauterine carcinosarcoma is a rare malignancy with about 30 cases reported in the literature [172–178]. Most tumors arise in the pelvic peritoneum, with occasional reports of tumors of the abdominal wall, colonic serosa, retroperitoneum, and omentum. There is a reported association with radiotherapy and BRCA mutations. An origin from the secondary Mullerian system is postulated; the tumors could arise directly from

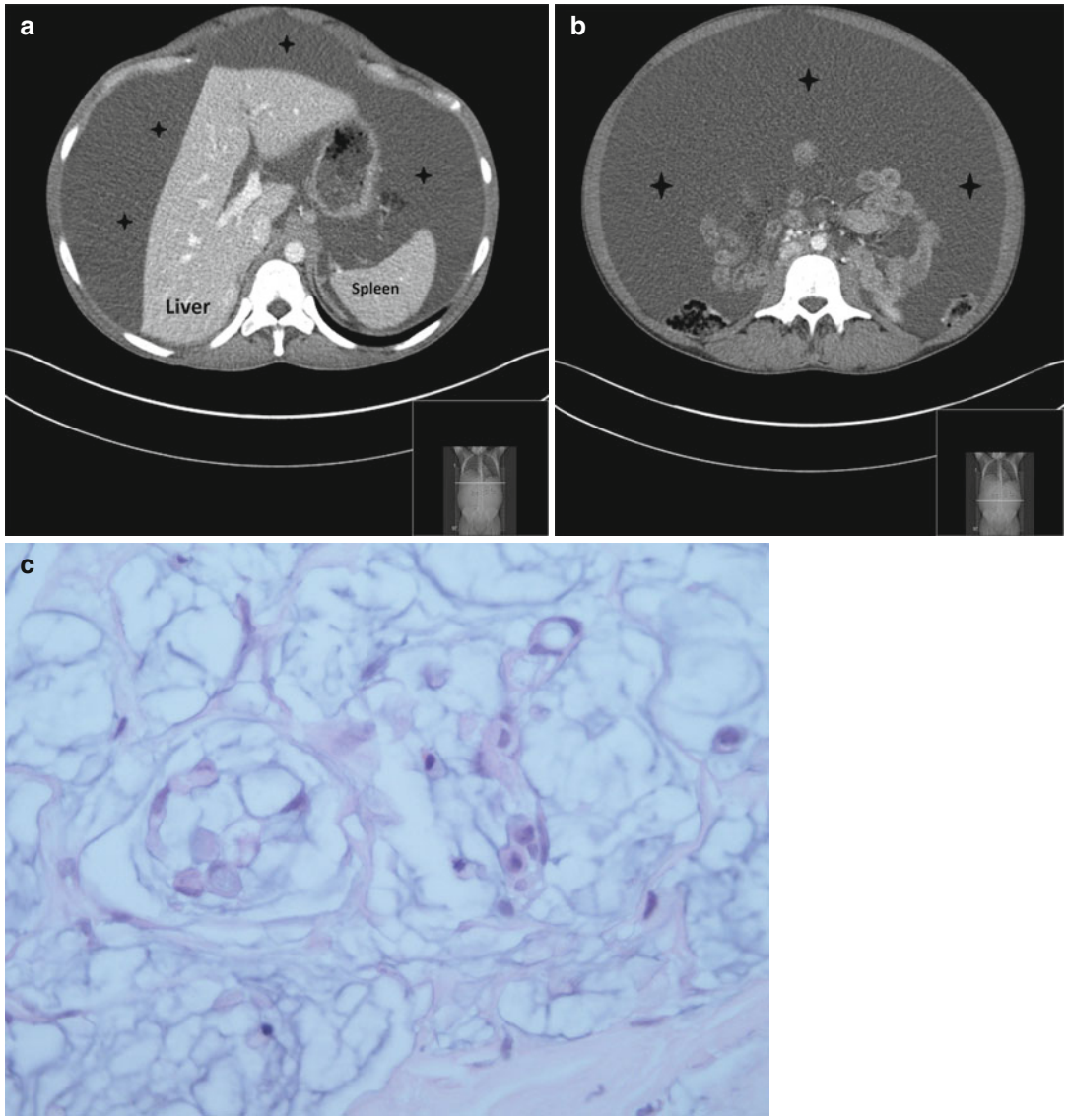

Fig. 18.17 (**a**) Pseudomyxoma peritonei. CT of the abdomen following intravenous contrast enhancement. Myxoid material scalloping the liver margin and displacing the spleen posteriorly (Courtesy of Dr Anju Sahdev). (**b**) Pseudomyxoma peritonei. CT of the abdomen following intravenous contrast enhancement. Myxoid material has displaced the bowel loops centrally and posteriorly in the abdomen, a typical mass effect appearance with pseudomyxoma peritonei (Courtesy Dr Anju Sahdev). (**c**) Pseudomyxoma peritonei (HE). The patient had diffuse peritoneal disease and carcinoma of the stomach. There were pools of mucin and signet ring cells on peritoneal biopsy

the coelomic epithelium or alternatively from foci of endometriosis or endosalpingiosis. It is now accepted that female genital tract carcinosarcomas (malignant mixed Mullerian tumors) behave biologically as epithelial malignancies. They are monoclonal in origin and should be regarded as metaplastic carcinoma rather than collision tumors or sarcomas [179–182]. The neoplasms consist of malignant epithelial and sarcomatoid elements and are positive for CK7 and vimentin. They can be differentiated from malignant mesothelioma by the absence of immunostaining for mesothelial markers such as calretinin or D2-40.

Adenosarcoma

These are rare Mullerian neoplasms comprising benign-looking glands lined by Mullerian-type epithelium in a malignant stroma that usually resembles low-grade endometrial stromal sarcoma. Occasional cases of sarcomatous overgrowth of the stroma have been reported [183–190].

Intra-abdominal Desmoplastic Small Round Cell Tumor (DSRCT)

This is an aggressive "small blue cell" tumor. It generally occurs in children or young adults with a distinct male preponderance (male to female ratio 4:1), but there are rare reports of occurrence in postmenopausal woman [191, 192]. The histogenesis of the tumor is uncertain, but it usually occurs on mesothelium-lined surfaces (predominantly within the abdomen) and is thought to be a primitive tumor arising from mesothelial cells or submesothelial mesenchyme. There is a reciprocal translocation [t(11;22) p(13;12)] that results in the expression of a chimeric fusion (EWS/WT1) transcript of the EWS1 gene on chromosome 22 and the Wilms' tumor suppressor gene on chromosome 11. The translocation is unique to this tumor and is found in all DSCRTs regardless of their location [193–195]. The Ewing sarcoma/peripheral neuroectodermal group of tumors have a [t(11; 22) (q24;q12)] translocation involving the long arm of chromosome 11, resulting in the EWS/ERG fusion gene; this gene transcript has been found in rare DSCRTs, and it is thought that there might be some overlap between these two groups of tumor [196].

The typical presentation is of an intra-abdominal mass with smaller tumor nodules studded throughout the peritoneal cavity. The neoplasm consists of islands of cells separated by desmoplastic stroma (Fig. 18.18a). The tumor cells have small, round to oval nuclei with scanty cytoplasm (Fig. 18.18b). Focal nuclear molding, frequent mitoses, and apoptosis are observed. Occasional intranuclear, eosinophilic, cytoplasmic inclusions may be present. These neoplasms are thought to show diverse differentiation, which is reflected in the immunoprofile. They may exhibit a tubular or trabecular architecture and rarely spindle cells with abundant eosinophilic or clear cytoplasm may be observed. This can give rise to a mistaken diagnosis of carcinosarcoma.

Immunohistochemistry: the neoplastic cells express low molecular weight cytokeratins (Cam 5.2), CD56, WT1, and EMA. There is dot-like cytoplasmic positivity for desmin and vimentin that corresponds to the whorls of paranuclear intermediate filaments seen ultrastructurally [197]. Variable positivity for SMA, CA125, S100, MOC31, NB84, BerEP4, chromogranin, and synaptophysin may be seen. Cytoplasmic CD99 reactivity is present in contrast to the membranous staining seen in Ewing sarcoma/PNET. HBA71 (Ewing sarcoma/PNET antigen) is negative [198–200].

The differential diagnosis includes other high-grade small blue cell tumors. Ewing sarcoma/PNET (Fig. 18.18c, d) shows a different pattern of CD99 positivity and lacks the desmoplastic stroma seen in DSCRT. Small cell and neuroendocrine carcinomas may appear similar morphologically, but lack dot-like desmin reactivity and occur in an older subset of patients. High-grade lymphoma (Fig. 18.18e) is excluded by an appropriate panel of lymphoma markers. Rhabdomyosarcoma is usually negative for cytokeratins. Wilms' tumor and neuroblastoma may appear similar and have a similar immunoprofile, but these tumors occur in young children, whereas DSRCT affects a somewhat older age group. Nevertheless, accurate diagnosis may well require chromosomal analysis.

Diffuse Peritoneal Leiomyomatosis

This is a rare condition which is usually reported in premenopausal women [201–205]. It is generally an incidental finding often discovered during Caesarean section. Small nodules are scattered randomly over the peritoneal surface. The nodules consist of smooth muscle cells, collagen, and fibroblasts (Fig. 18.19). During pregnancy or postpartum, decidual cells may also be present.

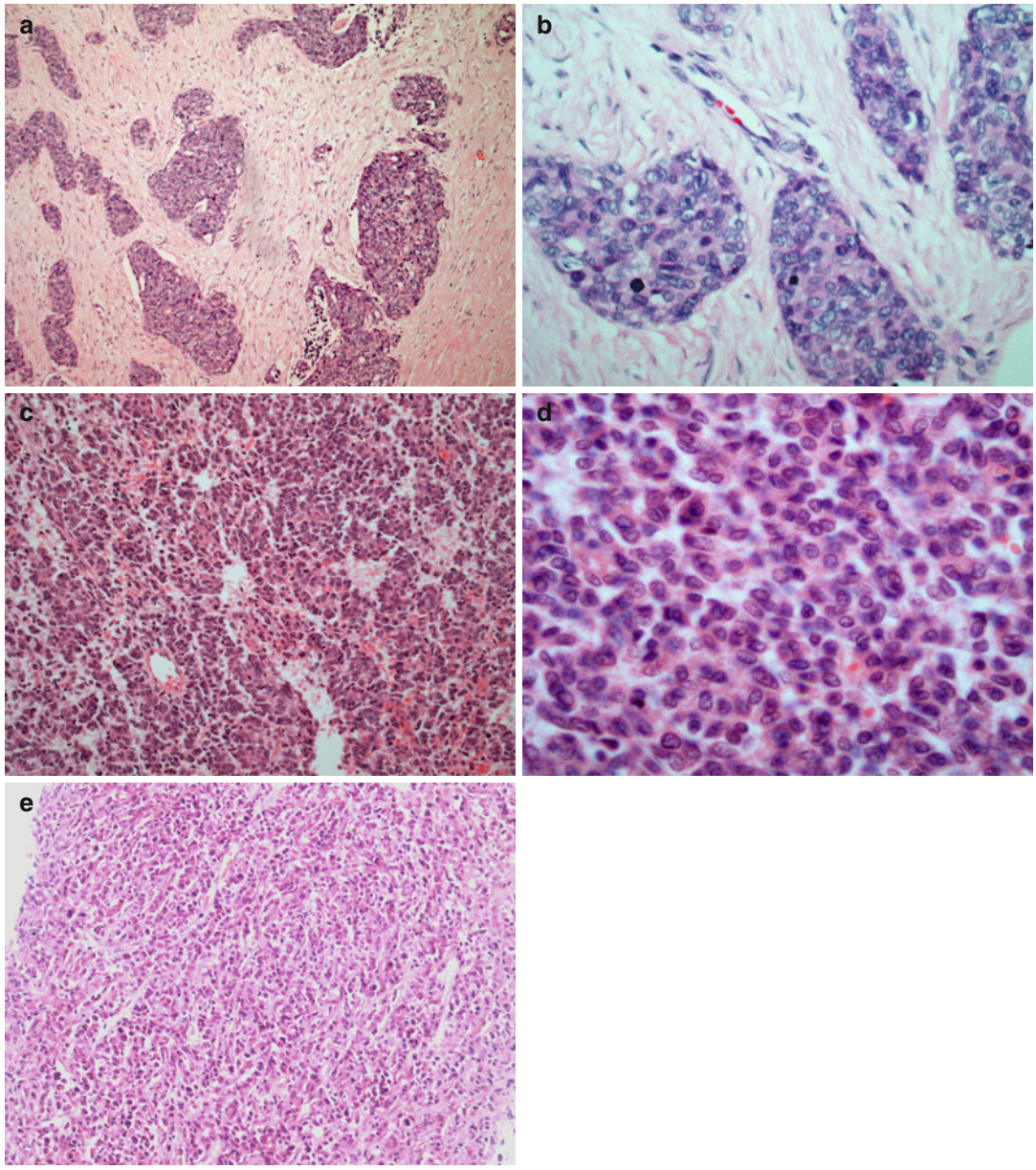

Fig. 18.18 (**a**) Desmoplastic small round cell tumor. (**b**) Desmoplastic small round cell tumor, scanty cytoplasm, frequent mitoses and apoptosis. (**c**) Differential diagnosis of small blue cell tumors – Ewing sarcoma/PNET. (**d**) Ewing Sarcoma/PNET. (**e**) Differential diagnosis of small blue cell tumors – diffuse large B cell lymphoma. The patient presented with diffuse peritoneal and nodal disease

The condition should be differentiated grossly and microscopically from disseminated spindle cell malignancies such as leiomyosarcoma. There is no cytological atypia and mitotic activity is inconspicuous. The tumorlets are hormone dependent and occur in hyperestrogenic states such as pregnancy, in association with a granulosa cell tumor or in women on oral contraceptives. There are usually associated uterine leiomyomata. It is regarded as benign and responds to GnRH agonists. Malignant transformation has been reported in a handful of cases [206].

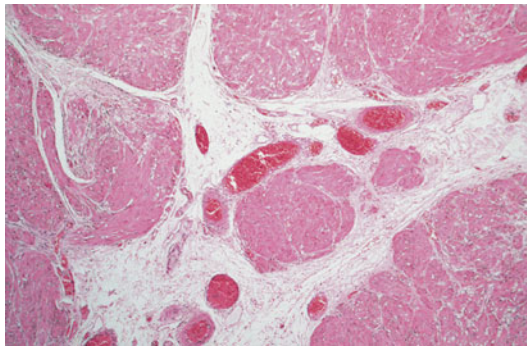

Fig. 18.19 Disseminated peritoneal leiomyomatosis. Bland spindle cells with no atypia or necrosis

Solitary Fibrous Tumor

These tumors commonly occur in the pleura but may sometimes involve peritoneal or retroperitoneal sites [207–210]. They are believed to originate in the submesothelial mesenchyme and are typically reactive for CD34. The behavior is usually benign although rare reports of sarcomatous transformation exist [211, 212]. They are well-circumscribed neoplasms composed of bland spindle cells in collagenized stroma. Various patterns are recognized: the hemangiopericytomatous pattern with staghorn vessels, the collagenized form with slit-like spaces, thick collagen and scanty tumor cells, and cellular lesions with a storiform arrangement. Immunohistochemically the cells are positive for CD34, vimentin, and bcl2. They are negative for cytokeratins, S100, and muscle markers such as SMA. The differential diagnosis includes gastrointestinal stromal tumor (GIST). This is discussed below. The differentiation from other spindle cell tumors found at this site includes leiomyoma (positive for SMA and caldesmon) and peripheral nerve sheath tumors (S100 positive).

Inflammatory Myofibroblastic Tumor

The usual site for this lesion is the lung, but extrapulmonary tumors have been reported [213–216]. There is a higher incidence in children and young adults and the presentation is of an abdominal mass with weight loss, fever, and blood dyscrasias such as anemia, thrombocytosis, and polyclonal hypergammaglobulinemia. The tumors consist of spindle cells often in a storiform or nodular fasciitis-like pattern, collagen, and a lymphoplasmacytic infiltrate. The cells are positive for vimentin, cytokeratin, and actin in keeping with the immunoprofile of myofibroblasts. Anaplastic lymphoma kinase (ALK-1) positivity is present in a proportion of these tumors and many of them express p53 [217, 218]. Coughlin et al. in their series of inflammatory myofibroblastic tumors discovered that ALK-1 negative tumors were more likely to be associated with adverse outcome compared to ALK-1 positive lesions.

Calcifying Fibrous Tumor (CFT)

This is a soft tissue lesion which usually present in the subcutis or deep soft tissues of the extremities and head and neck and trunk in children. A few cases have been reported in the pleura and the peritoneum [219–224]. The lesion consists of thick bundles of collagen often arranged in a whorled pattern with scattered fibroblasts, psammomatous calcification, and a lymphoplasmacytic infiltrate. The fibroblasts are positive for vimentin and negative for cytokeratin and ALK-1. CD34 reactivity is controversial; some authors maintain that these lesions are always CD34 negative, but there are also reports of CD34 positive tumors.

Gastrointestinal Stromal Tumor

This is not a peritoneal tumor but can present as an intra-abdominal mass. It is a neoplasm of uncertain malignant potential that is believed to arise from the interstitial cells of Cajal, which are pacemaker cells in the gut wall. It typically consists of spindle cells in a fascicular or storiform pattern. The cells are positive for CD117, DOG-1, and CD34 and can sometimes express smooth muscle and neural markers such as SMA and S100.

Synovial Sarcoma

This is an aggressive mesenchymal neoplasm with a characteristic chromosomal translocation t[(X;18)(p11;q12)] that results in fusion of the

SYT gene on chromosome 18 with the SSX1 or SSX2 gene on the short arm of the X chromosome. The tumor usually occurs in the extremities or the limb girdle, but there have been reports of primary tumor within the abdomen. It may occur in the retroperitoneum, the abdominal wall, or the pelvis [225–230]. Histologically, the neoplasm can be monophasic or biphasic. Biphasic tumors have sarcomatoid as well as epithelioid components, whereas in monophasic tumors one or the other of these components predominates (Fig. 18.20). The differential diagnosis includes carcinosarcoma for biphasic tumors, whereas the monophasic tumors need to be distinguished from malignant mesothelioma, desmoplastic small cell tumor, metastatic carcinoma, and other soft tissue sarcomas such as leiomyosarcoma, fibrosarcoma, and rhabdomyosarcoma. The neoplasms are usually reactive for broad-spectrum cytokeratins, EMA, CK7, and vimentin and a large proportion express calretinin.

Genetic studies and a full panel of immunostains may be required for diagnosis, including CD99, muscle markers (SMA, myoglobin), mesothelioma markers (calretinin and D2-40), broad-spectrum cytokeratins, and markers for small blue cell tumors (including CD99, NB84, CD3, CD20, low molecular weight cytokeratin, chromogranin, synaptophysin, and CD56). The peritoneum is an unusual site for synovial sarcoma and definitive diagnosis will probably require chromosomal analysis.

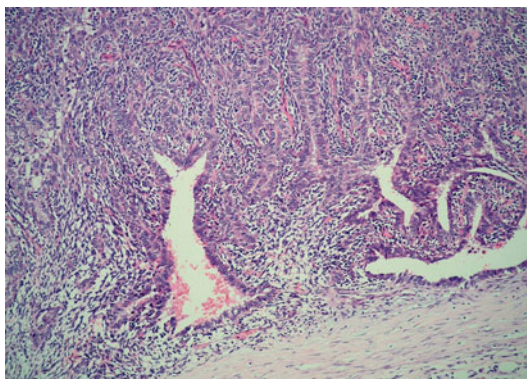

Fig. 18.20 Biphasic synovial sarcoma with epithelial and mesenchymal components (Courtesy of Prof. Glenn McCluggage)

Metastatic Disease

A number of metastatic neoplasms may present as intra-abdominal peritoneal masses. The primary tumor may be in the lung, breast, the gastrointestinal tract, urinary bladder, female genital tract, or hematoreticular system. The pathologist will be confronted with an omental or peritoneal biopsy in order to establish the site of origin of the primary tumor which will determine subsequent patient management. Morphology is the mainstay of histopathology reporting; nevertheless, in many circumstances, a panel of immunohistochemical stains is required; rarely chromosomal analysis may be indicated (see above). The biopsy tissue may be scanty and fragmented and it is prudent to request a number of unstained slides even if they are not subsequently needed. In some cases, especially in widespread supra and subdiaphragmatic disease, it may not be possible to assign a primary tumor site, and immunohistochemistry may only be able to establish the difference between carcinoma, sarcoma, and lymphoma. In such cases close clinicopathological correlation and pattern of disease spread may sometimes be useful.

For poorly differentiated tumors, an initial panel including broad-spectrum cytokeratins (MNF116 and AE1/AE3), BerEP4, EMA, calretinin, lymphoma markers (CD3, CD20, CD79a, CD5), vimentin, desmin, actin, and inhibin may indicate whether the neoplasm is a carcinoma, mesothelioma, lymphoma, sarcoma, or sex cord stromal tumor. Germ cell neoplasms should always be borne in mind when the patient is young (Oct3/Oct4, AFP, PLAP, CD117, CD30); melanoma (S100, HMB45, melan-A) is a great mimic of any tumor type. It may be necessary to pursue further immunohistochemical investigation with a second line of antibodies to enable further typing/characterization of the neoplasm.

Metastatic Adenocarcinoma

Female Genital Tract

Uterine endometrioid tumors – CK7 positive, CK 20 negative, ER, and PR usually positive but may be negative in high-grade tumors

Uterine serous carcinoma – CK7 positive, p53 positive, and ER/PR negative
Uterine clear cell carcinoma – may only express CK7
Ovarian serous carcinoma – CK7 positive, WT1 positive, ER usually positive, most tumors are high grade and express p53.

Upper Gastrointestinal Tract

CK7 positive, CK 20 and CDX2 focally positive or negative, PR negative. In the case of pancreatic neoplasms, ER and WT1 may be expressed and CK7 may not always be positive.

Lower Gastrointestinal Tumors

CK 20 and CDX2 positive, CK7 usually negative or focally positive. ER and PR are usually negative.

Breast

CK7 positive, WT1 and CK 20 usually negative, ER, PR, and Her2neu should be correlated with the immunoprofile of the breast lesion (if this is available).

Lung

TTF1 positive, CK7 positive, napsin positive, calretinin negative, D2-40 negative, WT1 negative.

Small Cell/Neuroendocrine Carcinoma

The pulmonary type is often positive for TTF1, Cam5.2 (dot-like cytoplasmic reactivity), CD56, chromogranin, and synaptophysin. The ovarian type, which occurs in young women, is associated with hypercalcemia and is usually TTF1 negative.

This list is by no means exhaustive and a small percentage of cells in any tumor may exhibit patchy immunoreactivity with antibodies that are not normally associated with them.

Benign Lymph Node Inclusions

Lymph node inclusions are not uncommon in the pelvic, para-aortic, and inguinal lymph nodes in women. They are usually discovered incidentally at Caesarean section or in the course of lymphadenectomy for regional neoplasia. They should be considered, especially when examining lymph nodes during intraoperative frozen section.

Mullerian Inclusions

These can be in the form of cysts or small glands. They are usually present in the capsular or interfollicular areas but can sometimes affect the entire lymph node [231, 232]. (The glandular lining consists of a single layer that is usually tubal in type with ciliated and non-ciliated cells, including intercalated cells. Apical mucin is often and rarely squamous metaplasia has been described [233].) The main differential diagnosis is metastatic adenocarcinoma; the lesions can be differentiated by the heterogeneity of the cells, lack of mitotic activity, and absence of cytological atypia. The situation can be complicated when found in association with serous borderline tumors; in such cases, the inclusions may exhibit atypical features and sometimes the distinction from metastatic carcinoma can be difficult or even impossible.

Mesothelial Inclusions

These usually occur in mediastinal lymph nodes but may occasionally be seen in pelvic nodes [234, 235]. They occur in the form of small glands or single cells, usually in the subcapsular area (Fig. 18.21a, b). The cells have dense cytoplasm and bland nuclear features.

Nevus Cells

There are numerous reports of nevus cells within lymph nodes, usually in the capsular region. In the context of female genital tract disease, the usual site is the inguinal region [236–239]. The cells may be mistaken for metastatic malignant melanoma or squamous cell carcinoma and should be borne in mind in particular when examining sentinel lymph nodes in vulval carcinoma. The cells are morphologically bland and mitotic figures are absent. Nevus cells express S100 and

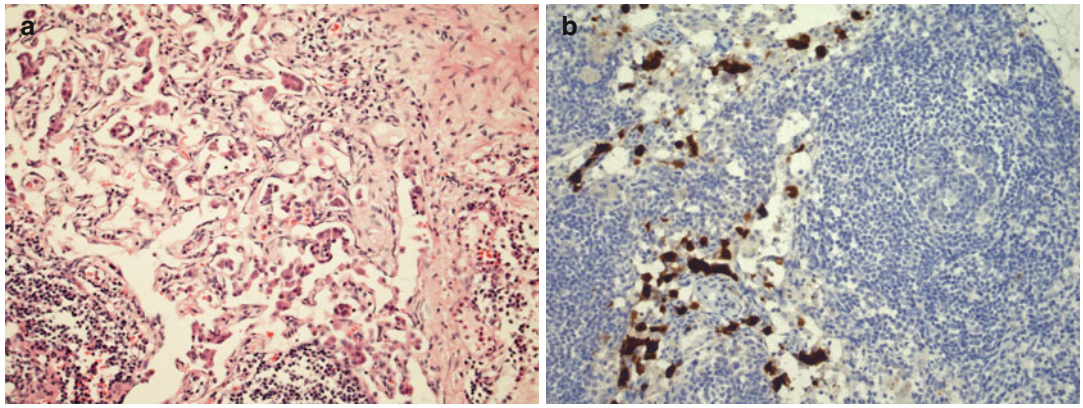

Fig. 18.21 (**a**) Benign pelvic lymph nodes inclusions – mesothelial cells. This was an incidental finding in lymphadenectomy for cervical carcinoma. (**b**) Mesothelial lymph node inclusions highlighted with calretinin

are usually negative for HMB45 and the proliferation index using Ki67 is low [240].

Ectopic Decidua

Decidual deposits in the abdominopelvic nodes may occur in the absence of endometriosis [241]. These have no clinical significance but should be differentiated from metastatic squamous cell carcinoma. Although the cells may appear cytologically atypical, there is no mitotic activity or keratinization present.

Intranodal Leiomyomatosis

This is generally seen in association with uterine leiomyomata or diffuse peritoneal leiomyomatosis. It is thought to be secondary to lymphatic spread from the uterine tumor, although some cases may arise from myofibroblasts that have undergone organization or trapped sub-coelomic mesenchymal cells [242–244]. The smooth muscle cells appear benign with a fascicular architecture and are positive for SMA and caldesmon, mitotic activity is absent, and Ki67 is low.

The differential diagnosis includes metastatic leiomyosarcoma (uterine mass, cytological atypia, and mitotic activity), lymphangioleiomyomatosis (usually associated with pulmonary lesions, HMB 45 positive), neural tumors (S100 positive), and Kaposi's sarcoma (HHV8 and CD34 positive).

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Mesenchymal Tumors of the Ovary 19

Paul A. Bennett

Abstract

This chapter deals with "pure" mesenchymal tumours of the ovary; a subset of rare ovarian tumours that are composed of mesenchymal tissue without an epithelial, germ cell or sex-cord stromal element. These tumours have an uncertain histogenesis, and the notion of a "pure" ovarian mesenchymal tumour is disputed for many of the entities described in this chapter.

Introduction

The wide variety of pure ovarian mesenchymal lesions reported in the global literature has led to controversy over the histogenesis of these rare lesions, primarily because the ovary bears only a limited amount of normally-occurring mesenchyme from which tumours can develop. For example, whilst it may be speculated that ovarian leiomyomas can arise from vascular or ligamental smooth muscle, the origin of apparently pure ovarian rhabdomyosarcomas is less clear cut given the absence of striated muscle in the normal ovary. Possible origins of mesenchymal lesions therefore include mesenchymal overgrowth of heterologous tumours, overgrowth of the stromal element of an endometriotic deposit, growth within a cyst wall, or origin from mesenchymal tissue within a Sertoli-Leydig cell tumour or a mature or immature teratoma. It therefore follows that each of these theories relies on the obliteration of other tumour components by the proliferating mesenchyme, in order for these lesions to appear purely mesenchymal. These probable pathways of histogenesis are yet to be convincingly proven, however, and the possibility of *de novo* mesenchymal neoplasms arising within the ovarian stroma has not yet been fully excluded.

What follows is a brief discussion of these rare tumours, including clinical presentation, morphological features and prognosis. We have not included pure fibrous tumours of the ovary within this chapter, on account of their presence in the continuum of fibroma-thecoma sex cord-stromal tumours. These entities are instead described in Chap. 15.

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Tumors of Smooth Muscle

Leiomyoma

Leiomyomas of the ovary are relatively uncommon, with up to 100 cases described in the worldwide literature [1–52]. An age range of 16–79 years has been reported. Although usually unilateral, bilateral ovarian leiomyomas appear to be more common at the younger end of the spectrum, with cases reported in 16- and 21-year-olds [16, 17, 22]. The most commonly described clinical features are nonspecific and include pain, abdominal swelling, and a palpable mass. Other presenting features include bilateral hydronephrosis [26], pleural effusion [10, 40], ascites [42], and virilization secondary to reactive hyperplasia of the theca interna [7, 13, 25]. Many ovarian leiomyomas were incidental findings, including one discovered during a termination of pregnancy [20]. They have been reported to have an appearance similar to uterine leiomyomas on dynamic-contrast MRI imaging [45].

Grossly, ovarian leiomyomas can vary in size, in keeping with their uterine counterparts. The reported maximum dimensions vary from 3 mm [18] to 250 mm [49]. They are typically solid and well circumscribed with a whorled, gray-white cut surface.

In the majority of reports, these lesions show the characteristic histological features of a leiomyoma: bundles and whorls of eosinophilic spindle cells displaying regular, blunt-ended nuclei. Rarely, they have been reported to show a prominent lipomatous component [9, 15]. Typically, the myocytes display minimal (insignificant) atypia and sparse or absent mitotic activity. As yet, an ovary-specific model of categorizing atypical leiomyomas does not exist due to the relative rarity of these lesions. In cases of increased cellularity and mildly increased mitotic activity, the terms "cellular leiomyoma" and "mitotically active leiomyoma" have been used, in keeping with the terms used for uterine lesions [38]. Similarly, pleomorphic leiomyomas that lack significant mitotic activity have been reported as "symplastic" or "leiomyoma with bizarre nuclei" [8, 38].

Immunohistochemically, ovarian leiomyomas show immunoreactivity for h-caldesmon, desmin, smooth muscle actin, and vimentin.

The differential diagnosis of leiomyomas includes fibro-thecomatous lesions, on account of their bland, fascicular, spindled morphology. However, these lesions lack the cigar-shaped nuclei of leiomyoma and often show more prominent extracellular collagen deposition. While they may show smooth muscle actin-positive immunoreactivity, fibro-thecomatous lesions are negative for desmin and h-caldesmon. Differentiation from leiomyosarcoma is discussed below. Finally, ovarian involvement by leiomyomatosis peritonealis disseminata and intravenous leiomyomatosis should be ruled out.

Surgical excision is recommended, with some authors advising preoperative frozen section and ovary-preserving surgery in reproductive age women [51, 52]. Limited evidence is available regarding prognosis, although no cases of recurrent ovarian leiomyoma have yet been reported, including in the mitotically active variants.

Leiomyosarcoma

Primary ovarian leiomyosarcoma is rare, with less than 30 individual case studies documented in the global literature [53–79], together with a larger series of 26 cases [38]. The tumors most commonly occur in postmenopausal women, though have been reported to occur in patients as young as 20 [74]. Common presenting symptoms are pain, abdominal swelling, and a palpable abdominal mass. They have been discovered in conjunction with ovarian leiomyomas, suggesting malignant transformation of these lesions [67].

Grossly, ovarian leiomyosarcomas tend to be large, solid masses that can measure up to 250 mm maximally [79]. They typically have a more variegated appearance than benign tumors,

showing areas of necrosis and hemorrhage. Microscopically, ovarian leiomyosarcomas are composed of interlacing bundles of plump spindle cells, as expected of smooth muscle tumors. A minority have been reported to be myxoid [64, 78] or epithelioid [75], in keeping with uterine leiomyosarcomas. Compared to leiomyomas, ovarian leiomyosarcomas show prominent nuclear pleomorphism and hyperchromaticity, together with prominently increased mitotic activity including atypical mitoses. Coagulative tumor cell necrosis is also sometimes encountered (Fig. 19.1). While the criteria for defining smooth muscle malignancy in the ovary are not widely defined, Lerwill et al. proposed that at least two of the following features should be present to secure a diagnosis of malignancy: moderate or severe diffuse cytological atypia, a mitotic count $\geq$10 figures/10 high power fields, or tumor cell necrosis [38]. However, it should be noted that the authors in this series warned that nuclear atypia and a lower mitotic count were sufficient for clinically malignant behavior in a minority of cases. As for uterine lesions, it is perhaps reasonable to use the term "smooth muscle tumor of uncertain malignant potential" in the small number of cases where the distinction of a benign from a malignant neoplasm cannot be made reliably on the presently available criteria.

Immunohistochemically, ovarian leiomyosarcomas typically show positive immunoreactivity for h-caldesmon, desmin, smooth muscle actin, and vimentin. Some studies report bcl-2 immunoreactivity in these lesions [72, 78], the significance of which is uncertain.

The main differential diagnosis for these lesions is metastatic leiomyosarcoma from elsewhere, particularly the uterus. This should be supported by a previous history, in particular of a sarcoma at another site or the presence of a uterine mass. These cases must be discussed at the gynecological oncology multidisciplinary meeting and, depending on local protocol, also at the sarcoma multidisciplinary meeting. Secondary involvement by gastrointestinal stromal tumors should also be considered. These are often less pleomorphic and less mitotically active than the usual leiomyosarcomas and show positive immunoreactivity for CD117 (c-kit) and CD34.

The recommended treatment of ovarian leiomyosarcoma is surgical debulking and a full staging laparotomy. There are few studies into the efficacy of chemoradiotherapy, although most reports suggest it is of unproven benefit [69, 71]. Follow-up of many of the above case reports is incomplete, but the overall prognosis for these lesions is bleak. Lerwill et al. reported a mortality rate of 62 % at a mean of 24 months for the cases in their study [38]. A similarly high mortality rate is reported for many of the other case reports in the literature.

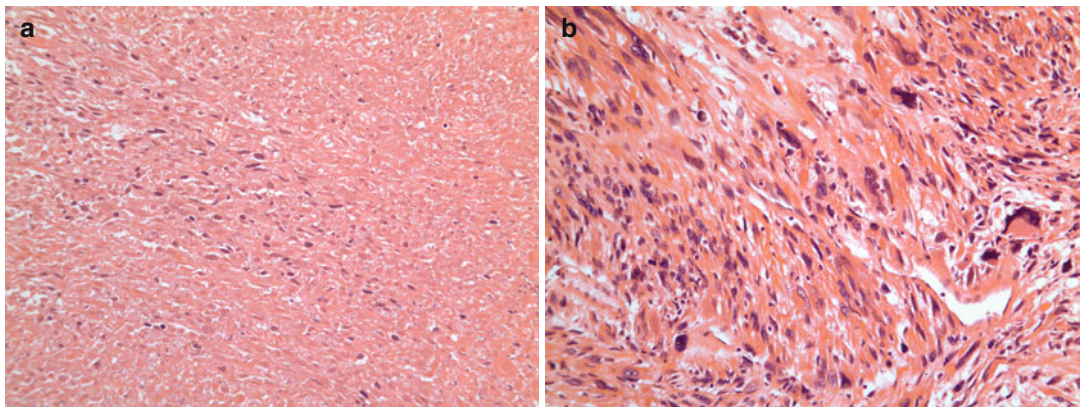

Fig. 19.1 (**a**) The typical pattern of coagulative necrosis seen in leiomyosarcoma, with admixed cytologically atypical myocytes. (**b**) Ovarian leiomyosarcomas show diffuse cytological atypia and increased mitotic activity, as seen here

Tumors of Striated Muscle

Rhabdomyoma

A single case of ovarian rhabdomyoma has been recorded in the literature. Rather than being a "pure" neoplasm, this case comprised small nodules of rhabdomyoma within the wall of a serous cystadenoma [80].

Rhabdomyosarcoma

Pure primary rhabdomyosarcomas of the ovary are rare, with less than 20 recorded examples [81–91] and a small series of 13 cases [92]. They have been reported between the ages of 4 and 79 years. Common presenting symptoms are abdominal pain and swelling, with many having evidence of metastatic disease at presentation. One case presented with such extensive bone marrow involvement, together with atypical cells in the blood, that it was initially diagnosed as an acute lymphoblastic leukemia [85].

Grossly, these lesions are unilateral and can measure up to 195 mm maximally. The cut surface varies from gray to yellow, with areas of hemorrhage and necrosis. Microscopically, the majority of the documented cases are of the embryonal subtype, which are typically composed of variably cellular sheets of small round blue tumor cells with little cytoplasm. These cells often show brisk mitotic activity. A clue to diagnosis is the presence of rhabdomyoblasts, which are typically larger with prominent eosinophilic cytoplasm. Less commonly seen are alveolar rhabdomyosarcomas, which form irregular nests of eosinophilic cells with round nuclei and prominent nucleoli.

Immunohistochemically, the tumor cells show strong positive immunoreactivity for myoglobin, myo-D1, myogenin, desmin, and smooth muscle actin. Electron microscopy may reveal Z-bands and myofilaments.

The differential diagnosis of embryonal rhabdomyosarcoma must include other small round blue cell tumors, especially in cases presenting in children. Lymphoma, leukemia, neuroblastoma, primitive neuroectodermal tumor, and small cell carcinoma of pulmonary or hypercalcemic type should all be considered. These may all present as primary or secondary lesions in the ovary. Alveolar rhabdomyosarcomas may need to be differentiated from metastatic epithelioid neoplasms, such as carcinomas and melanomas. The use of immunohistochemistry should render the diagnosis straightforward in most cases.

Most patients are treated with a full staging laparotomy including salpingo-oophorectomy and adjuvant chemotherapy. Ovarian rhabdomyosarcoma is an aggressive tumor with a poor prognosis, based on the limited prognostic data available. In the case series by Nielsen et al. [92], 8 of the 13 patients had extra-ovarian disease at presentation, and a total of 7 patients had died within 26 months. However, a recent case series showed a good initial response to vincristine, doxorubicin, and cyclophosphamide in children treated for both the embryonal and alveolar subtypes [89].

Tumors of Cartilage

Chondroma

While benign cartilaginous tissue is commonly found as a component of other ovarian tumors, pure chondromas are exceedingly rare. A single well-documented case exists, in which a 39-year-old woman presented with an ovarian tumor entirely composed of benign mature cartilage [93].

Chondrosarcoma

Primary chondrosarcomas of the ovary are also very rare, with only a single case report in the literature [94]. This was in a 61-year-old woman who presented with an abdominal mass. In this case, the chondrosarcoma appeared well differentiated and was successfully excised. There was no evidence of recurrence 4 years later.

Typically, chondrosarcomas have a blue-gray cut surface. Microscopically, the chondrocytes are more numerous, more pleomorphic, and more mitotically active than those seen in benign cartilaginous lesions. Immunohistochemistry is of little use.

The main differential diagnoses are between malignant cartilage in a carcinosarcoma with heterologous elements and metastatic chondrosarcoma from elsewhere. Thorough sampling of the lesion to look for epithelial elements, together with a good clinical history, is essential.

Tumors of Bone

Osteoma

There are no convincing examples of pure osteomas in the literature. Osseous metaplasia is a fairly common component of leiomyomas and fibromas, and osseous tissue is commonly seen in teratomas.

Osteosarcomas

Pure ovarian osteosarcomas are extremely rare, and only seven cases have been reported so far [95–101]. These are in the age range of 43–75 years and all presented with an abdominal mass and/or abdominal pain. A calcified mass is sometimes visible on abdominal X-ray [97, 98].

Grossly, these tumors usually measure over 100 mm in maximum diameter. The cut surface is solid and hemorrhagic, with focal areas of cystic change and necrosis also reported. Calcified areas may also be present, as described above. Microscopically, these tumors show the typical features of osteosarcomas seen elsewhere in the body: highly pleomorphic spindle cells with numerous mitotic figures arranged in sheets and bundles, associated with focal osteoid deposition.

Due to the typical appearance of osteosarcoma, the main differential diagnoses include metastasis from elsewhere and origin in a heterologous tumor. A careful history and extensive sampling should provide clues to the origin of this tumor.

As with other ovarian mesenchymal tumors, the limited data on pure ovarian osteosarcomas means that prognostic information is lacking. However, the studies quoted above suggest that these lesions are very aggressive, with most patients dying within 8 months. Hines et al. [97] report that their patient was disease-free following 8 courses of cisplatin-doxorubicin chemotherapy, but this is not reflected in any other studies.

Tumors of Neural Tissue

Neurofibroma

Pure neurofibromas of the ovary are extremely rare, and only two cases have been reported [102, 103]. Each of these was in a patient with known type 1 neurofibromatosis. One case presented as a pelvic mass, simulating a malignant neoplasm, while the other presented as chronic pelvic pain.

Like typical neurofibromas elsewhere, ovarian lesions are usually solid and well circumscribed. Microscopically, they are poorly defined and are composed of fascicles of elongated, tapering spindle cells with inconspicuous mitotic activity. The stroma may be focally myxoid and may also show scattered mast cells. The spindle cells are focally positive for S100, aiding in the main differential diagnosis with fibromas, which are S100 negative. The prognosis appears to be excellent, with no recurrence reported after 3 years in one of the above cases [103].

Schwannoma

Schwannomas of the ovary have been described [104] but are again extremely rare. They apparently resemble schwannomas seen elsewhere. In contrast to neurofibromas, they show a more varied hyper- and hypocellular sheetlike appearance (the so-called Antoni A and Antoni B areas) and are more diffusely and strongly S100 positive.

Ganglioneuroma

A single case of pure ovarian ganglioneuroma has been reported [105]. This tumor arose in a 4-year-old girl who presented with a swollen abdomen. Upon excision, the ovary was entirely replaced by ganglion cells, which typically show pale eosinophilic cytoplasm, round nuclei, and prominent nucleoli.

Malignant Peripheral Nerve Sheath Tumor

A well-described case of ovarian malignant nerve sheath tumor is reported in the literature [106]. This was diagnosed in a 71-year-old woman who presented with abdominal pain and swelling. A 15 cm smooth lesion was excised from the left ovary. Histologically, this tumor was extremely cellular, being composed of short fascicles of spindle cells with tapered nuclei and varying amounts of lightly eosinophilic cytoplasm. The mitotic count was 4 per 10 high power fields. S100 was negative, a reported feature in some malignant peripheral nerve sheath tumors [107]. This patient did not respond to doxorubicin and cyclophosphamide and died less than 5 months following diagnosis.

Paraganglioma

Paragangliomas of the ovary are rare. A recent small case series [108] included three patients of ages 22, 58, and 68 years. The ovaries were grossly solid, with tumor sizes ranging from 80 to 220 mm. The microscopic appearance was typical of paraganglioma, with each case showing a nested ("Zellballen-like") tumor of granular epithelioid cells set within a vascular stroma. The tumor cells stained positively for neuroendocrine markers, and one case showed the classical appearance of peripheral S100-positive sustentacular cells around the nests. The presence of focal inhibin positivity was raised as a potential pitfall in these lesions, particularly when considering sex-cord stromal tumors as a differential diagnosis. The typical features of paraganglioma are demonstrated in Fig. 19.2.

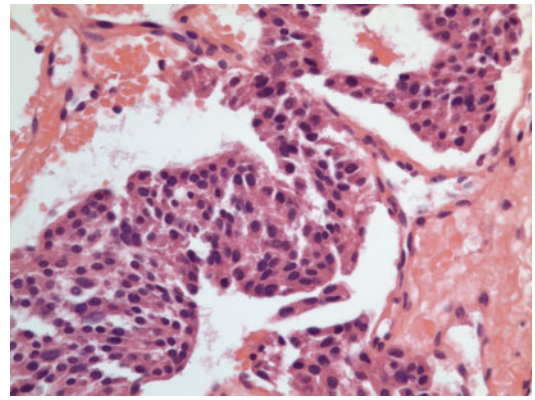

Fig. 19.2 Ovarian paragangliomas often form small nests, or "Zellballen," as seen here. A rim of sustentacular cells is visible surrounding the nested cells

Tumors of Vascular and Lymphatic Tissue

Hemangioma

The ovary has a rich vascular supply, and it is therefore unusual that ovarian hemangiomas are so rare, with only approximately 40 well-recorded cases in the literature [109–139]. These occurred between the age ranges of 11 and 81 years. Most presented with an abdominal mass or were discovered incidentally during surgery. Other means of presentation included ascites [126, 129, 136], acute abdominal pain [123], an elevated serum CA125 [129, 132, 136], and pleural effusion [132]. Most hemangiomas show a characteristic vascular pattern on ultrasonography, although markedly calcified hemangiomas have also been detected on preoperative imaging [138]. Bilateral lesions have occasionally been reported [119].

Grossly, hemangiomas are usually only 10–20 mm in maximum diameter. However, larger lesions of over 100 mm have been reported [127]. The cut surface is typically spongy and hemorrhagic. Microscopically, the diagnosis is usually straightforward. Most hemangiomas are of the cavernous type, showing variably sized

blood-filled spaces lined by a single layer of endothelial cells. Intravascular thromboses may be present. Adjacent stromal luteinization has been reported in a small number of cases [125, 126, 130]. Immunohistochemically, these lesions stain positively for CD31, CD34, and factor VIII-related antigen.

As always, the ovary should be extensively sampled to exclude a hemangiomatous component of a teratoma. Otherwise, the principal differential diagnosis lies between hemangioma and the small hilar capillary proliferations that are commonly seen in the normal ovary. This distinction can be difficult in smaller lesions, but the presence of a well-defined mass should secure the diagnosis of hemangioma. The absence of erythrocytes in the dilated spaces should raise the possibility of lymphangioma, which, unlike hemangioma, will show positive immunoreactivity for D2-40.

Ovarian hemangioma should be managed by simple oophorectomy.

Angiosarcoma

Pure angiosarcomas of the ovary are vanishingly rare, and around 20 well-reported cases are present in the literature [140–152]. Most arise in premenopausal women but have been described in patients as old as 81 years [151]. The most common presenting symptom is abdominal pain and swelling, although anemia associated with hematoperitoneum has also been reported [150].

Grossly, angiosarcomas can vary in size. A small series by Nucci et al. showed a range of 35–140 mm [144]. They are typically brown, hemorrhagic, and friable. Microscopically, they are composed of variably sized, proliferating vascular channels lined by markedly pleomorphic cells with brisk mitotic activity. There may be areas of solid growth and necrosis. Occasionally, a spindled morphology may predominate. Immunohistochemical reactivity for CD31, CD34, factor VIII-related antigen, and smooth muscle actin is typically encountered. Cytokeratins may be positive in epithelioid angiosarcomas.

Angiosarcoma may occur as part of a carcinosarcoma or an adenosarcoma, so an epithelial element should be searched for. In pure tumors, the main differential diagnoses lie between other malignant sarcomatous tumors, such as leiomyosarcoma, and metastases from elsewhere. Immunohistochemical immunoreactivity and a comprehensive discussion at the relevant multidisciplinary meetings will make the diagnosis clear.

These lesions are commonly treated with radical surgery and chemotherapy. Based on the limited examples available, the prognosis for angiosarcoma is poor, with many patients presenting with late-stage disease and dying within a year. However, a remission of 6 years has been recorded following treatment with doxorubicin and ifosfamide [148], and a case of apparent remission in a late-stage (FIGO IIIC) angiosarcoma has also recently been described following a six-cycle epirubicin and ifosfamide regimen.

Lymphangioma

Pure ovarian lymphangioma is very rare, and only a small number of cases have been reported [153–157]. They appear to arise in peri- and postmenopausal women, although one example was reported in a 19 year old following radiotherapy for Wilms' tumor [155]. The presenting symptom is usually an adnexal mass, although one case presented with chylous ascites [154].

Lymphangiomas can occur bilaterally [156]. Grossly, they display a honeycombed, spongy cut surface. Microscopically, thin-walled channels of varying sizes are seen, some of which may contain lymphocytes. The endothelial lining is flat and regular. Immunohistochemical reactivity for CD34, CD31, and D2-40 is usually seen.

The differential diagnosis lies between hemangioma and adenomatoid tumor. Distinction from hemangioma is discussed above. Adenomatoid tumors tend to contain solid cords of cells among their dilated channels and lack the typical immunohistochemical profile of lymphangioma. Instead, they show positive reactivity for mesothelial markers including calretinin and cytokeratins.

Tumors of Adipose Tissue

Lipoma

While fat is a common component of mixed tumors and teratomas, pure fatty lesions are extremely rare. An apparently pure lipoma was reported recently in a 66-year-old woman [158]. It was composed of sheets of benign fat, without any other tissue component.

Liposarcoma

A single case of pure ovarian liposarcoma has been reported [159]. It was diagnosed in a 13-year-old girl who presented with pelvic pain and a right ovarian mass. Histologically, the lesion was diffusely myxoid and contained a chicken-wire vascular morphology. The diagnosis of a myxoid liposarcoma was made. This was supported by demonstration of TLS-CHOP fusion by fluorescent in situ hybridization, which occurs as a result of the characteristic t(12;16) translocation associated with these lesions.

Miscellaneous Mesenchymal Tumors of the Ovary

Endometrioid Stromal Sarcoma

Primary ovarian endometrioid stromal sarcoma is again another rare tumor. Approximately 20 well-documented cases are described [160–165]. These arose in women between the ages of 20 and 76 years, who presented with symptoms of pain and abdominal swelling.

Grossly, the tumors measure an average of 110 mm [161]. Bilateral involvement is common. They are often solid and yellow-brown but may show areas of cystic change. Histologically, the appearance is comparable to endometrioid stromal sarcoma in the uterus. The typical pattern is sheetlike growth of oval and spindle-shaped cells with hyperchromatic nuclei and scant cytoplasm. Mitoses are generally inconspicuous. Small arterioles are commonly seen, around which the tumor may grow in a whorled pattern (Fig. 19.3). Areas of sex-cord stromal differentiation, smooth muscle differentiation, and myxoid change may occur. In contrast to uterine endometrioid stromal sarcoma, ovarian lesions tend to show a more nodular growth pattern and commonly lack the classical tonguelike growth pattern seen so often in the uterus. Endometriosis may be seen alongside these lesions and they may even originate within the stroma of an endometriotic cyst.

Immunohistochemically, ovarian endometrioid stromal sarcomas show positive reactivity for CD10, vimentin, and smooth muscle actin. Areas of smooth muscle differentiation may show positive reactivity for desmin and areas of sex-cord-like differentiation for inhibin.

The main differential diagnosis of ovarian endometrioid stroma sarcoma is a metastasis from the uterus. The absence of a uterine mass and the presence of endometriosis in the ovary are supportive of a primary ovarian lesion. Other possibilities include sex-cord stromal tumors and fibro-thecomas. In these instances, extensive sampling should identify at least some areas of more typical endometrial stromal differentiation. Furthermore, fibromas lack the typical vasculature of endometrioid stromal sarcoma. Care should be taken with immunohistochemistry for the reasons noted above and because some sex-cord stromal tumors also stain for CD10 [166].

Treatment is usually surgical, with some cases receiving chemotherapy and/or radiotherapy. The apparently successful use of hormone therapy has also been documented [164]. Overall, however, the outcome is unpredictable. The only large case series in the literature showed a correlation with tumor mitotic count. Of the 14 patients in this study with primary ovarian disease, 11 were still alive at the time of write-up. Those that died all had more than 10 mitotic figures per 10 high power fields [161].

Myxoma

Less than 20 ovarian myxomas have been reported [167–173]. The patients ranged in age from 12 to 46 years old and presented with abdominal or adnexal masses.

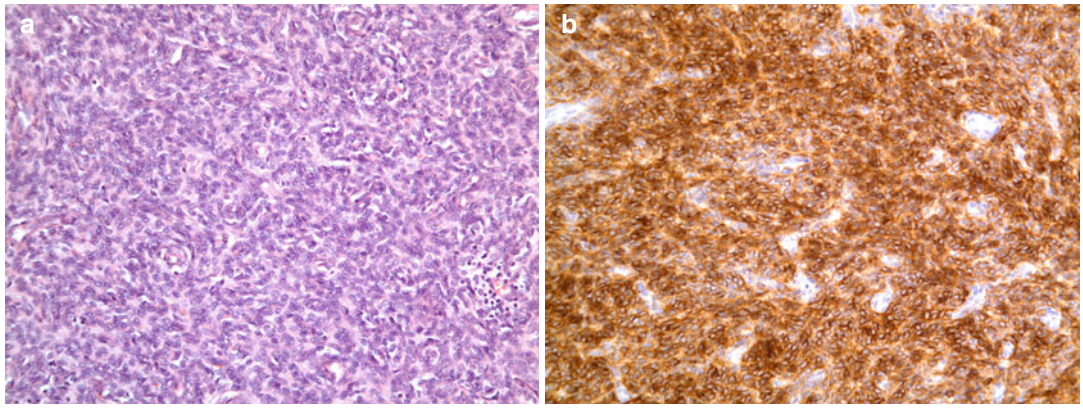

Fig. 19.3 (**a**) Endometrioid stromal sarcomas arising in the ovary are identical to their uterine counterparts, showing hyperchromatic cells arranged in a whorled pattern around small blood vessels. (**b**) CD10 positivity in endometrioid stromal sarcoma arising in the ovary

Macroscopically, these tumors ranged in size from 50 to 220 mm. Most appeared solid and gelatinous on cut surface, although some showed focal cystic change [169] and others had focal hemorrhagic features [171]. Microscopically, these lesions are composed of bland, variably hyperchromatic spindle and stellate cells with inconspicuous mitotic figures. These cells are dispersed within a loose myxoid stroma containing varying amounts of intervening capillary channels. Foci of fibrosis are occasionally seen (Fig. 19.4).

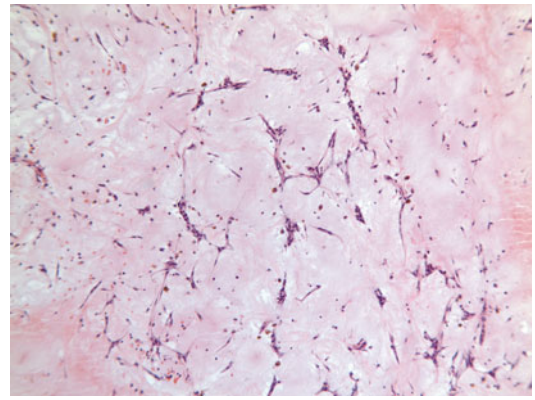

Fig. 19.4 The typical appearance of ovarian myxoma: spindle and stellate cells scattered in a loose myxoid stroma, together with intervening capillary channels

Myxomas have a nonspecific immunohistochemical appearance and typically show positive reactivity for vimentin and smooth muscle actin [169]. S100, cytokeratins, and vascular markers are negative. Desmin is usually negative, although focal positivity has been reported [170]. Staining for alcian blue is usually strongly positive, on account of the large amount of hyaluronic acid within the stroma of these lesions.

The differential diagnosis of ovarian myxomas includes massive edema of the ovary, which can be identified by the presence of normal ovarian structures within the stroma. The presence of prominent areas of fibrosis may point to an ovarian fibroma with myxoid change. Most importantly, myxomas must be differentiated from myxoid sarcoma, namely, liposarcomas or rhabdomyosarcomas. Extensive sampling of these cases should reveal some of the more typical features of these lesions, as described earlier in this chapter. Finally, epithelioid tumor cells within a myxoid stroma may point to a primary or metastatic mucinous carcinoma, which should also be excluded.

The treatment of ovarian myxoma is surgical excision. The prognosis is good, and all 13 patients in a small case series were tumor-free after 1–13 years [169]. A single case of recurrent disease is documented in a 65-year-old woman, 19 years after surgery [170]. This tumor had an aneuploid cell population and may have therefore represented a low-grade sarcoma as opposed to a typical myxoma.

PEComa

Perivascular epithelioid cell tumors (PEComas) have been the subject of much discussion in the 20 years since they were first identified as a unique group of tumors by Bonetti et al. [174]. The distinctive cells present in these tumors were named "perivascular epithelioid cells," a cell type which has no normal tissue counterpart. It was discovered that tumors of this cell type share common histological and immunohistochemical features, as described later. The PEC tumor group brought together angiomyolipomas of the kidney, clear cell ("sugar") tumors of the lung, and lymphangioleiomyomatosis. Since their initial description PEComas have been described in many different organs and tissues, including the ovary, despite some ongoing controversy over their histogenesis and nomenclature.

PEComas of the ovary are extremely rare, with only five cases reported in the literature. These include a 33-year-old woman with pulmonary and extrapulmonary lymphangioleiomyomatosis, the latter involving the ovary [175], and an epithelioid angiomyolipoma of the ovary associated with a separate renal angiomyolipoma in a 39-year-old woman [176]. Also described are a 41-year-old woman with cervical PEComa and intra-abdominal PEComatosis that involved the ovarian hilum [177] and a 59-year-old woman with malignant uterine PEComa and lymphangioleiomyomatosis affecting multiple sites, including the ovary [178]. Finally, the most recent example is of a 33-year-old woman with no significant history, who presented with an isolated ovarian PEComa [179]. Three of the above cases were in patients with known tuberous sclerosis [176–178].

Grossly, ovarian PEComas are reported to range from less than a millimeter in size (the so-called "PEComatosis," associated with larger lesions elsewhere) to up to 45 mm. The gross appearance was of a solid and/or cystic mass. Histologically, PEComas are typically composed of a mixture of spindle and epithelioid cells that display granular to clear cytoplasm and well-defined cell membranes. Nuclei are often regular, but bizarre forms have been reported [176]. Occasional nucleoli may be seen and mitotic figures are typically scanty. A perivascular distribution may be evident. Angiomyolipomas contain smooth muscle, prominent vessels, and admixed fat, along with the epithelioid cells mentioned above.

PEComas share a common immunohistochemical feature, in that the epithelioid cells are typically immunoreactive for melanocytic markers such as Melan-A and HMB45. Smooth muscle actin and vimentin are also commonly positive. Cytokeratins and desmin are negative.

The differential diagnosis of PEComas is relatively wide, and these lesions are often only considered following exclusion of other tumors. Their epithelioid and spindled morphology should necessitate exclusion of carcinomas and sarcomas. Recognition of some of the common morphological features of PEComa in the absence of a clear diagnosis should prompt the pathologist to request the appropriate immunohistochemical marker study, as described above.

Ovarian PEComas have all been treated with surgical excision. While most appear to behave in a benign fashion, it is difficult to speculate on the prognosis of these lesions, given the limited data available. A patient with widespread PEComatosis with ovarian involvement was disease-free 29 months following surgery [177]. It has been suggested that PEComas behave more aggressively if focally necrotic and of a large size. Whether or not this rule can be applied to ovarian PEComas remains to be seen. It is extremely likely that the coming years will produce more reports of this unique tumor type within the ovary.

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Specimen Cut-Up

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Abstract

Specimen cut-up forms an important part of the diagnostic process in the histopathological assessment of gynecological pathology specimens. While in small biopsy specimens there may be less diagnostic information that can be gained from macroscopic examination, in larger specimens, in particular, resection specimens, the macroscopic assessment is highly pertinent both diagnostically and as part of the information required to adequately and correctly stage the cancer. Unlike the microscopic examination, which will have the glass slides and paraffin-embedded tissue stored as part of the diagnostic record, specimens are generally disposed after a period of time following cut-up. This will vary in individual laboratories depending on the availability of storage space, but on average, the "wet" specimen is expected to be retained for at least 6 weeks following cut-up. It is important that if the case is considered to be unusual and may require the pathologist to return to it, at this stage, the specimen container should be marked with a "please keep/do not throw out" sticker. The macroscopic report with any photographs may be the only record that is kept of a specimen's macroscopic features. With this in mind, it is very important to perform a good macroscopic assessment and cut-up of specimens.

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Introduction

Specimen cut-up forms an important part of the diagnostic process in the histopathological assessment of gynecological pathology specimens. While in small biopsy specimens there may be less diagnostic information that can be gained from macroscopic examination, in larger specimens, in particular, resection specimens, the macroscopic assessment is highly pertinent both diagnostically and as part of the information required to adequately and correctly stage the cancer. Unlike the microscopic examination, which will have the glass slides and paraffin-embedded tissue stored as part of the diagnostic record, specimens are generally disposed after a period of time following cut-up. This will vary in individual laboratories depending on the availability of storage space, but on average, the "wet" specimen is expected to be retained for at least 6 weeks following cut-up. It is important that if the case is considered to be unusual and may require the pathologist to return to it, at this stage, the specimen container should be marked with a "please keep/do not throw out" sticker. The macroscopic report with any photographs may be the only record that is kept of a specimen's macroscopic features. With this in mind, it is very important to perform a good macroscopic assessment and cut-up of specimens.

Clinical Information and Patient Consent

The specimen request form and specimen container whether it be a pot or bucket must be checked by the pathologist for the patient demographic details, the laboratory number, and the specimen part, which must tally with the request form. In cases of a presumed malignant neoplasm, a staging laparotomy will include total abdominal/laparoscopic hysterectomy and bilateral salpingo-oophorectomy, omentectomy, lymphadenectomy, and peritoneal biopsies. An appendix is often performed, and segments of either the small or large intestine may also be submitted depending on the particular tumor and its involvement of the intestine. Rarely, a splenectomy specimen may also be submitted. Peritoneal washings and diaphragmatic scrapes may also be taken. These may be sent directly for diagnostic cytopathology analysis.

The pathologist, at the time of cut-up, should have all the clinical information and the radiological findings made available to them. A summary of the MDT's preoperative diagnosis is essential and the tumor marker profile should also be included. This may be submitted on the clinical request form or may be available to the pathologist on the IT system unique to the particular hospital and department.

The specimen request form is the means of communication between the surgeon and the pathologist. If there have been problems during the laparotomy, particularly in relation to capsular integrity, i.e., preoperative capsular breach or leakage, which may be identified by the surgeon during the operation, the pathologist should be informed. Any such areas are best photographed upon receipt of the specimen so that a record is retained which also helps to remind the pathologist of the specimen macroscopy when reporting. Where frozen sections are routine, these issues should have been dealt with at the time of frozen section when the capsular status should be meticulously recorded.

If the specimen is an "IDS" (interval debulking specimen) following three cycles of chemotherapy, then the pathologist should be aware. There may be little residual tumor, and examination of the omentum usually reveals firm areas which histologically are seen to be areas of fibrosis, calcification, and inflammation with or without residual tumor. (See below.)

If the specimen is being examined fresh in order to freeze tissue and retain as part of a tissue-banking protocol, it is essential that the ovarian capsule is examined and the patient consent checked prior to tissue sampling.

Tissue Preparation Prior to Cut-Up

Specimens should be in suitably sized containers and be immersed in an adequate volume of formalin to aid fixation. The recommended specimen to

formalin ratio is 1:20, but 1:10 is what is expected for adequate fixation. This is often not achieved. The specimen container should be "topped up" with formalin on receipt. Specimens should be transferred to larger containers if the original container is too small.

Prior to cut-up, specimens need to be fixed, although fixation will continue after cut-up while tissue in cassettes is in formalin. Some specimens will require "opening" at least one day prior to cut-up; in particular, a uterus will require slicing permitting fixation of the endometrium. Some pathologists may wish to slice large ovarian masses prior to cut-up to allow fixation. If so, careful macroscopic assessment of the specimen including recording the status of the ovarian capsule, measurement in three dimensions, and specimen weight and recording the nature of the contents of any cysts is required at the time of specimen "opening." There are differing practices with regard to slicing of fresh specimens to aid fixation, with only some pathologists adopting this. In view of the importance of describing the capsular surface and other features of ovarian masses discussed below, specimen photography can be useful to record the macroscopic findings in detail as a supplement to the macroscopic report. As tissue has different morphologies when fresh and fixed, examination of the specimen when unfixed as well as after fixation may be helpful (Fig. 20.1).

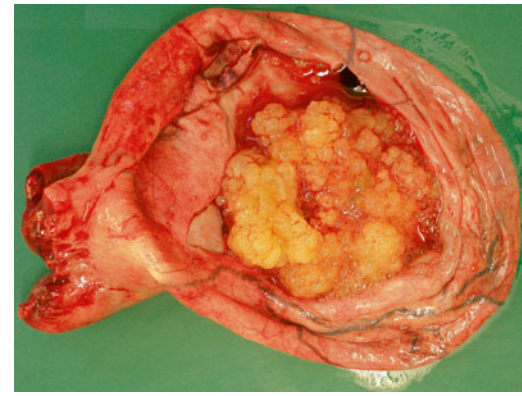


Fig. 20.1 This ovarian tumor mass (a serous borderline tumor) has been opened while fresh, revealing yellow papillary tissue within a unilocular cyst cavity. The capsule surface is pink and glistening unlike in the fixed state where tissue tends to take on a darker gray or brown color

At this stage, it may be considered appropriate to "ink" the capsular surface. If this is done, the capsular surface should be dried thoroughly with paper towels and then ink suitable for use in tissue processing should be applied by a paint brush on the capsular surface. Acetic acid should be used to "fix" the ink. This method has an advantage if trainees are cutting up the specimen, in which case it is easier to see which blocks represent capsular blocks even if a record has not been kept. Capsular breach is also easy to identify. The disadvantage is the obscuring of the Fallopian tube by ink. The pathologist must make a conscious effort to sample the Fallopian tube and therefore seek it out. An alternative to inking the entire capsule surface is to limit inking of the capsular surface to pieces of tissue taken for embedding. Inking with differential colors at the edges of defects in the capsule and of tumor on the capsular surface may also be helpful (Fig. 20.2).

Macroscopic Dissection/"Cut-Up"

The first stage of specimen cut-up should always include specimen identification checks. This should include checking patient demographic details and hospital identification details such as hospital numbers as well as the laboratory number assigned to the individual case by the histopathology department. Additionally, the specimen type should be checked, e.g., left ovary and Fallopian tube. The histopathology request card and all specimen containers need to be checked carefully for the above details, including checking that all labels on the specimen containers correspond with that on the request card. Either at the time of printing cassettes or prior to placing cassettes into formalin for processing, the laboratory number and any additional printed codes need to be carefully checked. Cut-up should not proceed until any discrepancies with the specimen identification checks have been investigated and corrected.

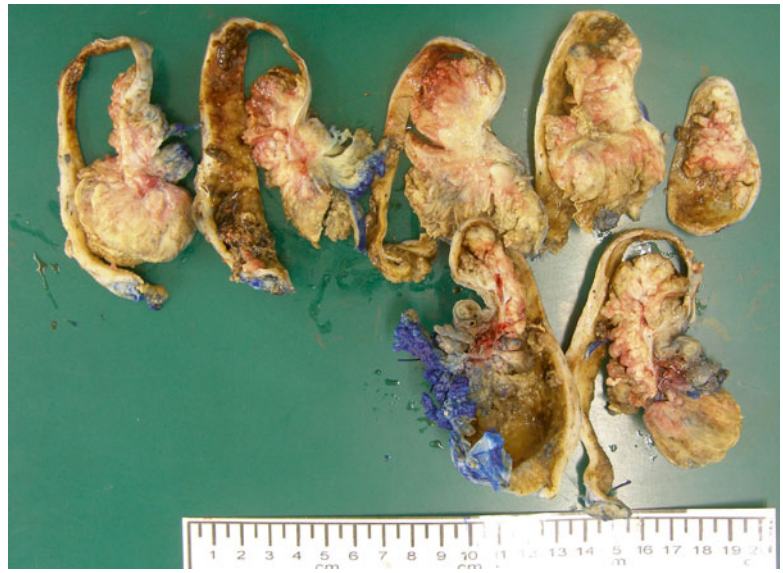


Fig. 20.2 This ovarian tumor (endometrioid adenocarcinoma) is partially cystic and partially solid. *Blue ink* has been applied to the tumor on the capsule surface to aid microscopic assessment of the capsule surface which is involved by tumor

At the time of cut-up, each histopathology case that is assigned a laboratory number is dictated separately often using a digital dictation system. This will clearly depend on the facilities at the individual laboratory. An audio cassette dictation system may also be used or a member of the laboratory staff may transcribe the dictation. A set order for dictation may be chosen by a department, such as, laboratory number, patient demographic details, date taken, clinical details, specimen type indicated on specimen containers, and finally, the macroscopic description. Various cut-up aids may be helpful, such as proformas, Royal College of Pathologists datasets and cancer staging systems.

The macroscopic assessment of a specimen should be dictated and subsequently edited to make a succinct macroscopic report which is relevant to diagnosis and staging. Pathologists may wish their macroscopic report to follow an anatomical sequence or alternatively the report may start with the most diagnostically relevant aspect of the specimen. Photographs can form an important part of the macroscopic diagnostic record for two principal reasons: (1) They can illustrate the macroscopic diagnostic and staging features of a specimen. (2) They can serve as a block key for the pathologist if block numbers are indicated on the photograph. Following on from this, it is important that a block key is recorded indicating the anatomical site of each block. This is the recommendation in the Royal College of Pathologists dataset [1]. The block key can be recorded at the end of the macroscopic report or if recorded elsewhere; it should be available to facilitate review of the slides in the future by any pathologist.

It is important to avoid "carry over" of tissue from other specimens or from different anatomical sites of the same specimen. This can be minimized by regular cleaning of the cut-up board and instruments. Paper towels should be used to dry the cut-up board and instruments rather than cloths. In addition, only one specimen container should be dealt with at a time. Avoidance of multiple specimen containers (even from the same patient) being present on the cut-up board while cutting-up one specimen may help reduce the risk of "carry over" or inadvertently sampling tissue from the incorrect specimen container.

Ovary Biopsies

Core biopsies of the omentum, transvaginal biopsies of ovarian masses or wedge biopsies of the ovary may be received by a histopathology

laboratory. These biopsies should be described briefly including basic measurements, prior to submitting all tissue for embedding and microscopic examination with sections at multiple levels following the protocols at individual laboratories.

Ovarian Cysts in Fragments

Ovarian specimens may be received as fragments of cyst wall if an ovarian cyst is removed laparoscopically. In this case, the outer capsular surface and inner cyst lining surfaces should be inspected, prior to embedding samples on edge.

Macroscopically Normal Ovaries as Part of Hysterectomy

Macroscopically, normal ovaries may be received as part of resection specimens, when generally one or two blocks may be sufficient. Blocks should be taken along the short axis of the ovary to include sections of the Fallopian tube. If they are taken as part of a malignancy from elsewhere in the gynecological tract other than the ovary, then three blocks from each side to include the Fallopian tube (or embedding all ovarian and Fallopian tube tissue) are preferred by the authors. This is not evidence based but anecdotal, as ovarian metastases of endometrial carcinoma have been identified microscopically in macroscopically normal ovaries using this approach.

Bilateral Salpingo-Oophorectomies

These patients have a family history of breast and ovarian carcinoma, and the surgery is performed as a risk reducing salpingo-oophorectomy. Some patients may have BRCA1 or BRCA2 mutations. The ovaries should be measured in three dimensions, any macroscopic abnormalities noted, sliced along their short axis, and embedded in their entirety. The Fallopian tubes should be measured and then embedded according to the SEE-FIM protocol given below and in Chap. 17 (Table 20.1).

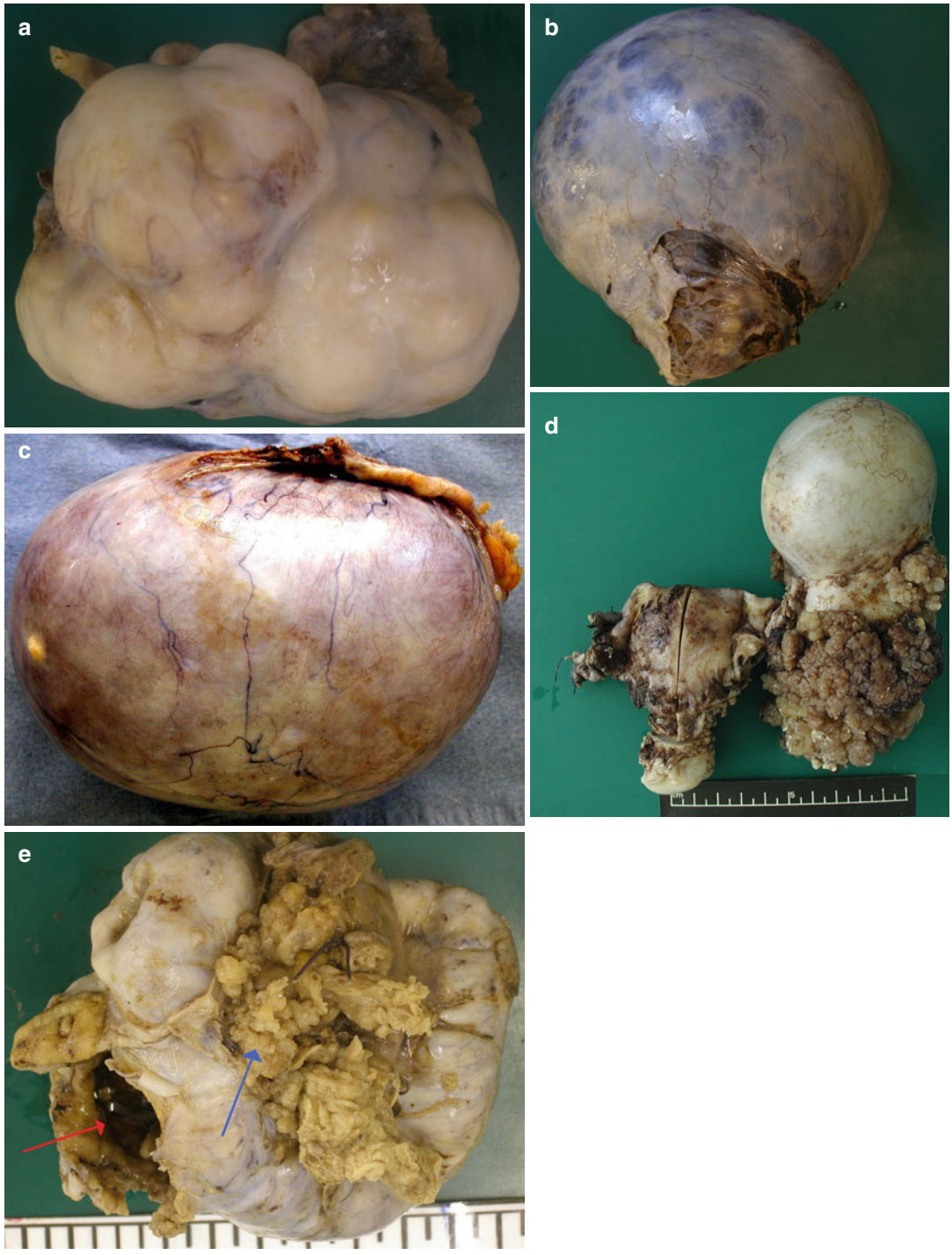
Table 20.1 The SEE-FIM protocol for examining Fallopian tubes in prophylactic salpingo-oophorectomies

1.	Tubes and ovaries are fixed in formalin for 1–2 h to reduce the risk of exfoliation during sectioning
2.	The distal 2 cm of the tube are amputated and sectioned sagittally into four sections
3.	The remainder of the tubes are sectioned at 2–3 mm intervals
4.	The ovaries are sectioned at 2–3 mm intervals
5.	The entire tube and ovary are submitted for histological analysis
6.	Areas of mucosa containing discrete loss of cilia with atypia are immunostained for both p53 and MIB-1 at the discretion of the pathologist
7.	In the event that special stains are used to verify a diagnosis of intramucosal carcinoma, the diagnosis must be corroborated by a second observer before issuing a pathology report

Ovarian Masses

Ovarian masses should be examined carefully prior to any dissection with particular attention to the ovarian capsule. The pathologist should carefully inspect the capsule for evidence of tumor on the surface and, in addition to any evidence of defects in the capsule, the presence of adhesions or capsular thickening associated with changes of color, as these may indicate issues with capsular integrity. This is clearly the single most important factor that will determine if the patient is to have further chemotherapy or not. It is important in staging the ovarian tumor (Fig. 20.3). Intraoperative rupture of the capsule may upstage an ovarian tumor. The significance of any defects in the capsule may require discussion with the surgical team as described above.

After macroscopic examination of the capsular surface, examination of the Fallopian tube, and any inking if undertaken, the ovarian mass should be sliced serially at 1 cm intervals and the internal nature of the ovarian mass or lining if the mass is cystic should be noted. Particular attention should be given to whether there are any solid areas or cystic locules. The cut surfaces should be assessed in detail to determine if it is a cystic lesion, a solid lesion, or a combination of the two. The number and range of diameters of cystic locules should be noted. It is important


a
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to identify and open all locules to allow complete examination and accurate diagnosis of the specimen. This includes apparently benign ovarian masses such as endometriotic cysts. The contents of the locules should also be described, which may be diagnostically helpful, such as in the case of mucinous tumors and endometriotic cysts which contain mucinous secretions and altered blood, respectively (Fig. 20.4a, b). The contents of mature cystic teratomas (hair with thick proteinaceous and oily material) are also diagnostically helpful being characteristic features of these tumors (Fig. 20.4c). The nature of the cyst lining should be described, including if the lining is smooth which would favor a benign epithelial neoplasm and if there are any papillary excrescences or solid areas which could suggest the possibility of a borderline or even a malignant component (Fig. 20.5).

Identification of solid areas macroscopically is also of diagnostic importance, in particular, in epithelial tumors, as the solid regions are the most likely site for carcinoma within the ovarian mass (Fig. 20.6). The presence of hemorrhage or necrosis in an ovarian mass should also be documented. At the appropriate age, a hemorrhagic and necrotic mass may indicate a yolk sac tumor (Fig. 20.6i).

Solid areas and papillary areas should be the focus for sampling. Extensive sampling of these regions is required as they are most likely to contain carcinoma or borderline tumor, respectively. However, sampling of representative areas of the ovarian mass should be performed including thin-walled cysts with smooth linings. If the mass is homogenous, then representative sampling is adequate in the first instance with the option of returning to the specimen should something untoward be identified on histological examination. The authors are aware of no clear evidence for how many blocks to take to ensure adequate sampling of neoplastic ovarian masses. However, some authors have suggested (at least) one block per cm of maximum ovarian tumor diameter [1, 2]. The Royal College of Pathologists (London, UK) has recommended the non-evidence-based practice of one block per cm of solid or papillary area for areas under 10 cm and two blocks per cm for larger lesions [3]. Others have suggested that one block per 2 cm of maximum ovarian tumor diameter is sufficient sampling [4]. More generous sampling may be advisable in the case of mucinous tumors as their benign, borderline, and carcinoma components can be extremely heterogeneous [5]. If the mass is grossly heterogeneous, then more extensive sampling is recommended avoiding obviously necrotic areas which are unlikely to be helpful. Sampling should be biased predominantly around the capsule as this is the area that is likely to be best fixed, in addition to most informative for staging purposes.

Tumors with exophytic components on the capsular surface should be extensively sampled. These include the borderline serous tumors that are most likely to show a micropapillary architecture and have associated "implants" (Fig. 20.3d). Multiple blocks of the capsular surface are

Fig. 20.3 (**a**) A 7-cm-maximum-diameter lobulated ovarian tumor mass. The capsule is smooth surfaced and glistening with no evidence of surface tumor or defects. The tumor was confirmed to be a benign Brenner tumor. (**b**) A 17-cm-maximum-diameter ovarian tumor with an intact capsule over most of the specimen with a smooth surface. There is also a defect visible in the capsule which exposes the underlying tumor (clear cell carcinoma). (**c**) A 21-cm-maximum-diameter ovarian tumor with a smooth capsule surface and Fallopian tube attached. The capsule is smooth and intact. There are no defects or areas of tumor involvement of the capsule. The ovarian mass was shown to be a borderline serous tumor with microinvasion. (**d**) A 14-cm-maximum-diameter ovarian tumor with attached Fallopian tube and uterus. The ovarian tumor is a borderline serous tumor and has a large area of papillary tissue visible on the capsule surface which represents involvement of the capsule by borderline serous tumor. (**e**) A 9-cm-maximum-diameter ovarian mass (endometrioid adenocarcinoma). There is a smooth capsule in some areas. However, in one area, there is yellow papillary tissue, representing involvement of the capsule by endometrioid adenocarcinoma (*blue arrow*). There is also a full thickness defect in the wall of the capsule exposing the lumen of a cyst cavity within the mass (*red arrow*)

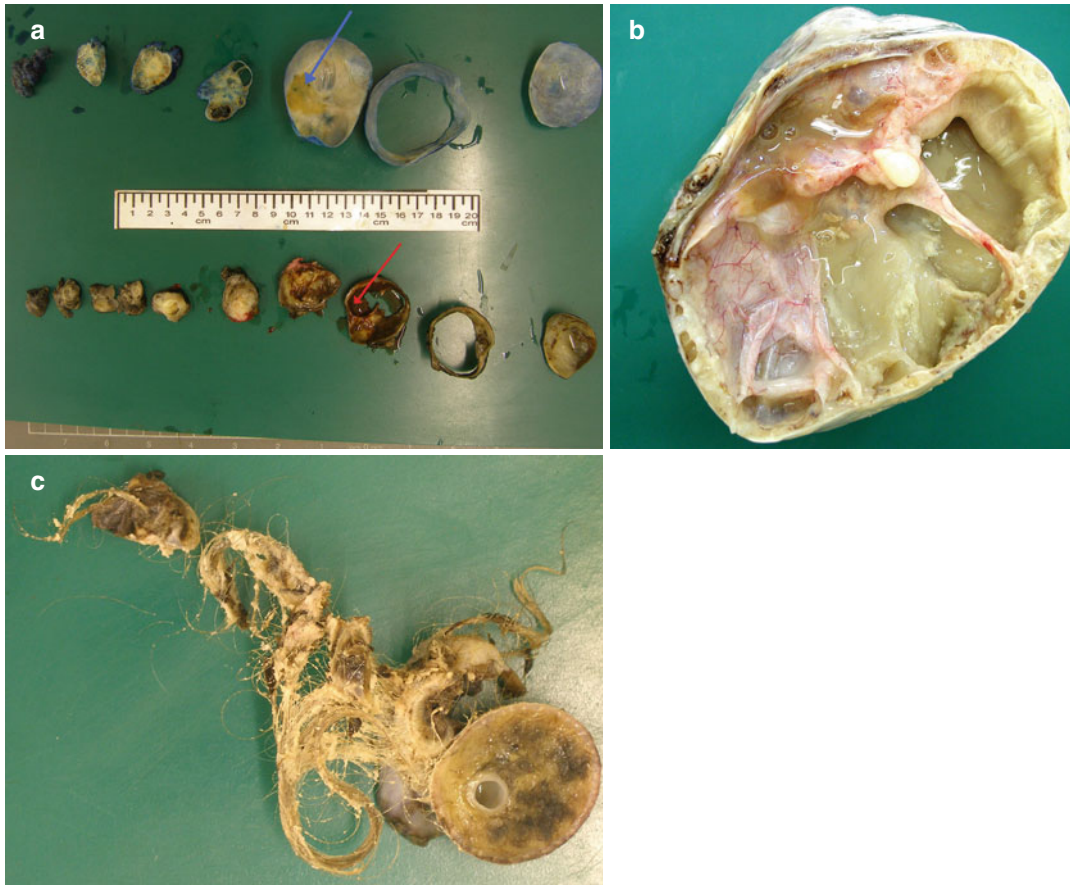


Fig. 20.4 (**a**) At the top of this figure is a unilocular mucinous cyst which contained thick yellow mucoid material, a small amount of which remains in the cyst cavity (*blue arrow*). The bottom of the figure shows a unilocular endometriotic cyst from the contralateral ovary of the same patient which contains thick brown altered blood (*red arrow*). (**b**) A 17-cm-maximum-diameter, multicystic ovarian tumor (borderline mucinous tumor) containing thick mucinous material within the cystic cavities. (**c**) A mature cystic teratoma characteristically containing hair with thick yellow proteinaceous and oily material

recommended, sampling any thickened areas or areas with adhesions as these are most likely to harbor the "auto-implants."

The contralateral ovary should be sampled in a similar manner if it also contains a tumor. If it appears normal, some authors have recommended one or two blocks [1]. However, some pathologists embed the entire contralateral ovary if macroscopically normal in borderline and malignant ovarian tumor cases. Again there is no evidence base to provide the definitive answer. The authors tend to sample most or all of the contralateral ovary and Fallopian tube if macroscopically normal.

Hysterectomy Specimens

The uterus removed with ovarian tumors should be measured in three dimensions and weighed if the local protocol indicates. The uterus should be examined in a similar manner to benign hysterectomy specimens. However, particular attention should be given to the uterine serosal surface for the presence of deposits of tumor that may be identifiable macroscopically. Any serosal tumor deposits should be sampled. Standard blocks should be taken including the anterior and posterior cervix and at least two blocks of the endometrium and myometrium including the full thickness

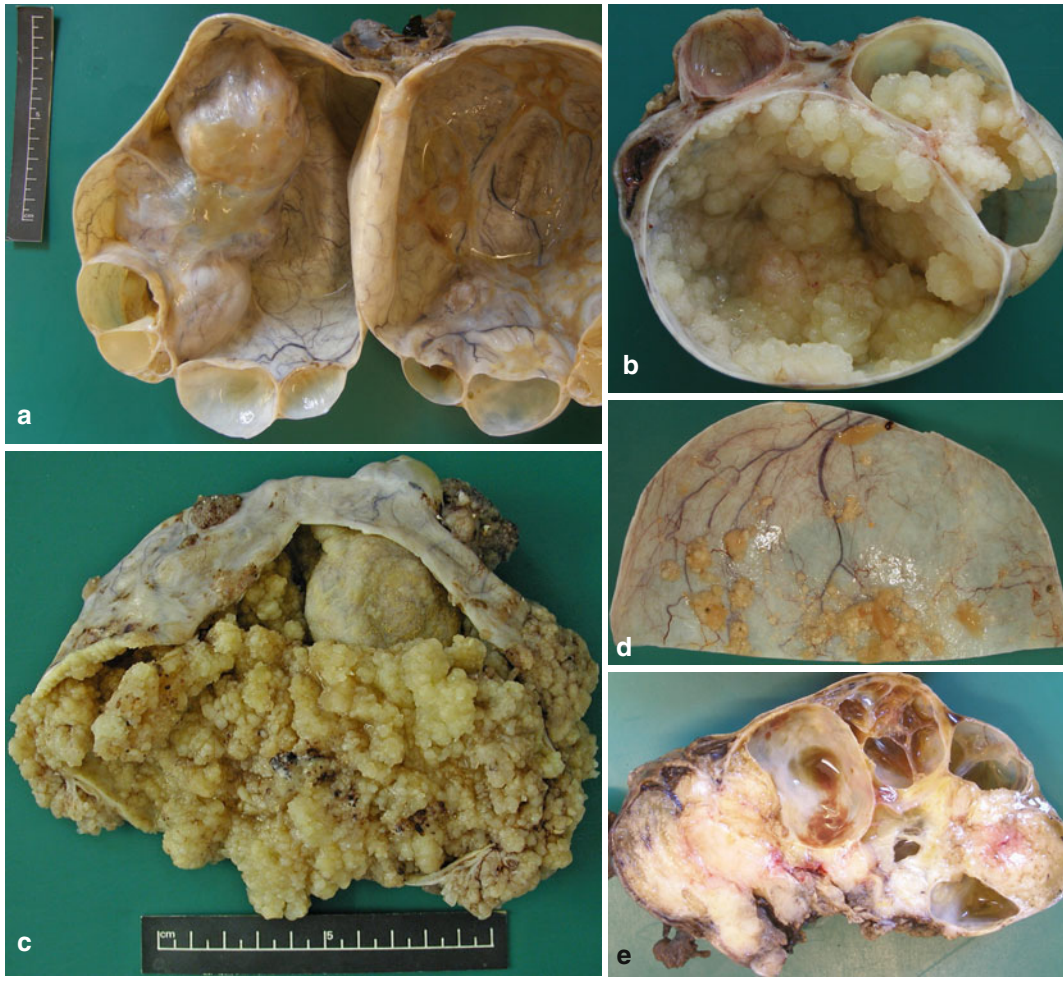
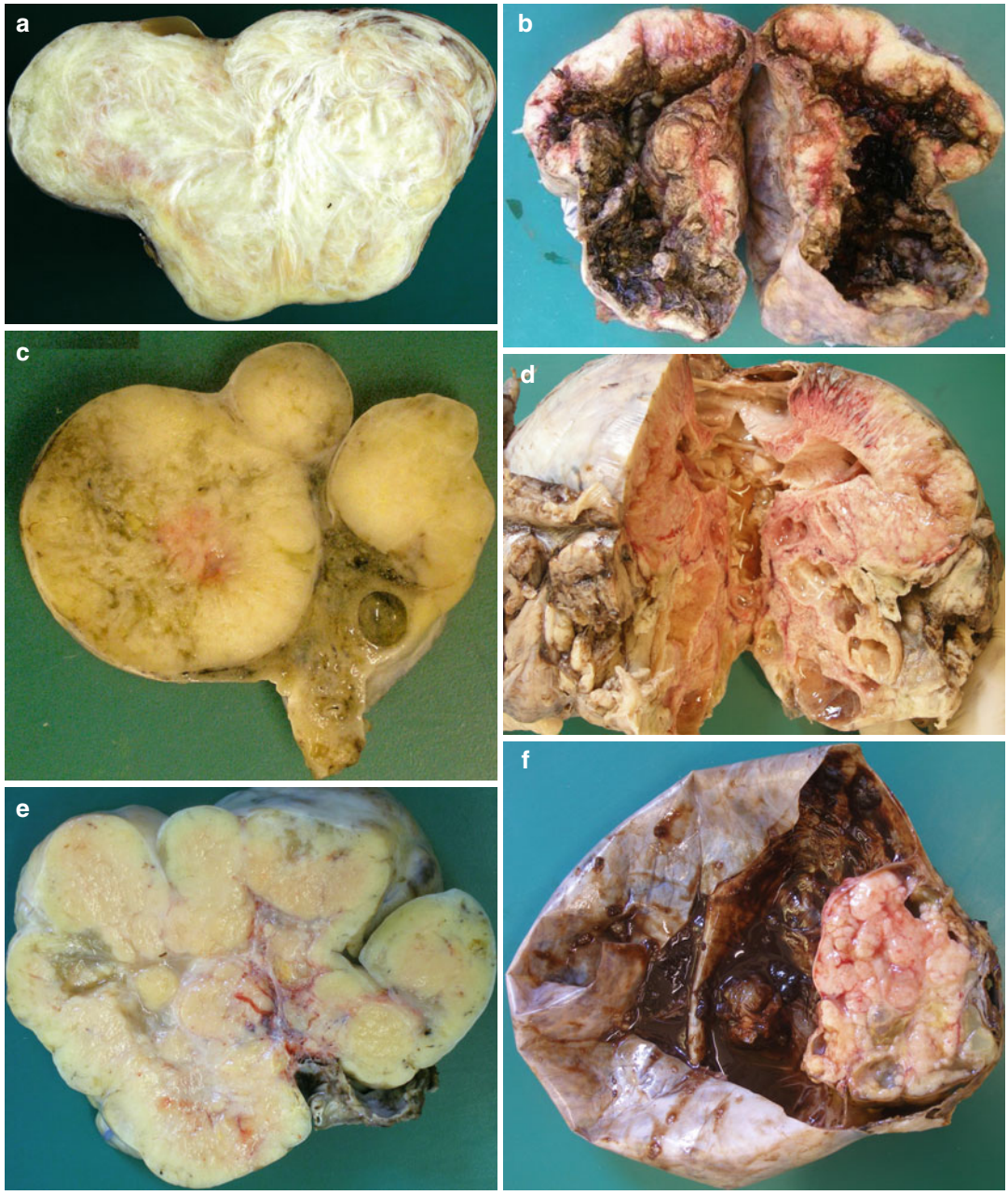


Fig. 20.5 (**a**) A benign mucinous cystadenoma. This is a multicystic ovarian tumor comprising cyst cavities with a wide variation of sizes, each of which is lined by smooth epithelium with no papillary areas or solid areas. (**b**) This is a borderline serous tumor of the ovary which has multiple cyst cavities containing abundant pale yellow papillary tissue. The papillary tissue represents the borderline serous tumor areas. The cyst cavity areas lined by flat epithelium represent benign areas. (**c**) This is a borderline serous tumor of the ovary which has been opened to reveal a large cyst cavity containing a mass of pale yellow papillary tissue. Also note that the capsule surface has areas of papillary tissue involving it. The papillary tissue represents the borderline serous tumor areas. (**d**) This is the lining of a large cyst cavity of an ovarian mass with multiple pale yellow areas of papillary tissue, representing borderline serous tumor. Histological analysis also revealed microinvasion, something that cannot be inferred from the macroscopic examination. (**e**) This 13-cm-maximum-diameter ovarian tumor has been sectioned to reveal areas of pale yellow solid tissue, in addition to cystic areas with variably sized cyst cavities. The solid tissue represents carcinoma. Histologically, the tumor had areas of endometrioid and serous carcinoma

of myometrium. Additionally, blocks of the pouch of Douglas, serosa at the posterior aspect of the cornua and parametrium are advocated to search for tumor deposits, as these sites are most commonly involved when serosal tumor deposits are noted. If the endometrium is thickened, this may represent endometrial hyperplasia which is a possibility if the ovarian tumor is a granulosa cell tumor producing estrogen. There may even be a synchronous endometrial carcinoma. Under these circumstances, the protocol for an endometrial carcinoma should be followed, and if histologically confirmed, the appropriate endometrial carcinoma dataset should be completed.

Fallopian Tubes

Fallopian tubes are commonly removed with ovaries and uterus. The Fallopian tubes received with ovarian tumors should be examined macroscopically for evidence of tumor and sampled including the fimbria, being a suggested site of origin of pelvic serous carcinomas [6, 7]. It is unclear how many blocks to sample of the Fallopian tubes in cases of borderline and malignant epithelial ovarian tumors.



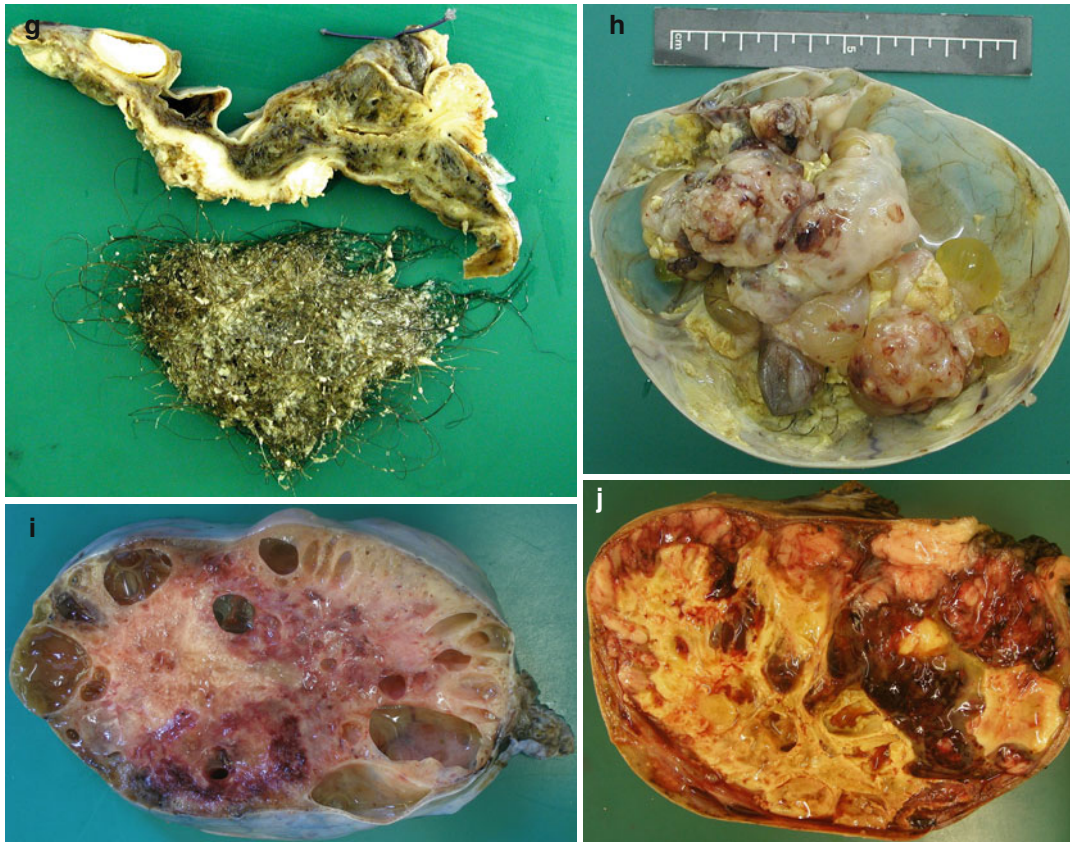


Fig. 20.6 (continued)

Fig. 20.6 (**a**) This is a 7-cm-diameter ovarian mass with a very firm white cut surface that appears whorled. The tumor is solid without cystic areas. The overall appearances are fairly typical for a fibroma. (**b**) This 16-cm-maximum-diameter tumor has been sliced to reveal a dominant cyst cavity which has a thick cyst wall with solid tissue within the wall which macroscopically suggests carcinoma. There are no thin-walled cysts or fine papillary areas that, if present, would suggest benign and borderline areas, respectively. Microscopy confirmed this to be clear cell carcinoma. (**c**) A 5-cm-maximum-diameter ovarian mass with a solid cut surface of pale yellow tissue, representing carcinoma (endometrioid, serous, and clear cell carcinoma). (**d**) A 21-cm-maximum-diameter ovarian tumor (metastatic colorectal adenocarcinoma) with a predominantly solid cut surface, in addition to cystic areas. (**e**) An ovarian tumor with a solid appearance on sectioning (metastatic breast carcinoma). (**f**) A 17-cm-diameter ovarian mass comprising a large endometriotic cyst containing altered blood. There is also an area of soft pale yellow solid tissue with some small cystic areas to the right of the figure which represents clear cell carcinoma. (**g**) A 10-cm-maximum-diameter ovarian cyst containing hair suggesting mature cystic teratoma. This mature cystic teratoma has white solid tissue in the cyst wall which represents invasive squamous cell carcinoma. (**h**) An immature teratoma present as a partially cystic, partially solid ovarian tumor mass. Immature teratomas are predominantly solid tumors, but cysts may be present as in the present case. (**i**) Yolk sac tumor. A 17-cm-maximum-diameter ovarian mass with a partially solid yellow and partially cystic cut surface, which is typical for yolk sac tumors. (**j**) Sertoli-Leydig cell tumor with heterologous elements. This 17-cm-maximum-diameter ovarian mass has a predominantly solid yellow cut surface with hemorrhagic areas, which is typical for Sertoli cell tumors and Sertoli-Leydig cell tumors. Cystic areas are also noted; a feature that is more common in Sertoli-Leydig tumors with heterologous elements

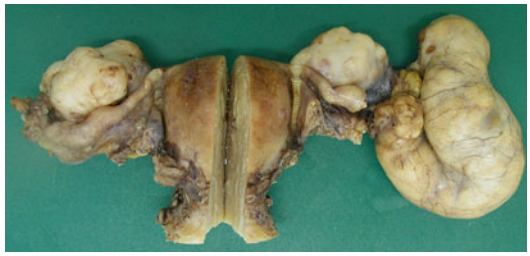


Fig. 20.7 Fallopian tube carcinoma. The left Fallopian tube is dilated up to 3 cm in diameter by Fallopian tube carcinoma and contained firm pale solid tumor on sectioning

However, some pathologists embed all Fallopian tube tissue bilaterally. The authors suggest embedding the Fallopian tube using the SEE-FIM protocol in these cases (Table 20.1) [8]. Uncommonly, a specimen may be received with a primary Fallopian tube carcinoma (Fig. 20.7).

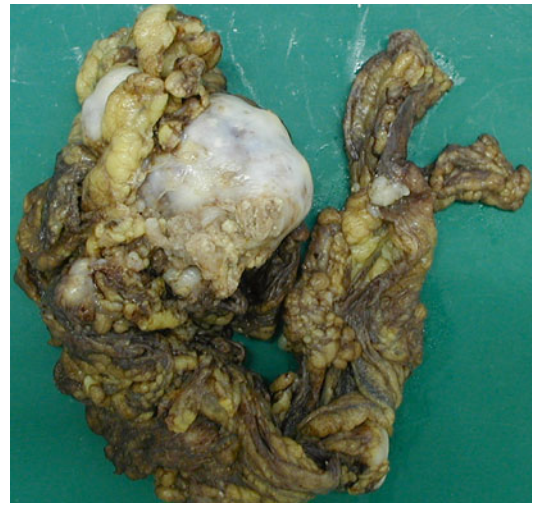


Fig. 20.8 Omentum containing a 7-cm-maximum-diameter deposit of firm white tumor (metastatic carcinoma from ovarian carcinosarcoma)

Omentum

An infracolic omentectomy is part of the staging procedure for ovarian carcinoma. The omentum should be measured in three dimensions and if local protocol indicates, weighed. Detailed macroscopic examination of the omentum is very important to detect tumor deposits and to allow accurate staging. The omentum should be inspected and palpated for evidence of tumor (Fig. 20.8). Whereas invasive carcinoma deposits may be palpable, implants associated with serous borderline tumors will generally not be palpable. Additionally, the omentum should be serially sliced at intervals as close as practically possible to aid detection of very small deposits. It is suggested that 1 cm intervals are appropriate. The maximum diameter of the largest tumor deposit should be measured as it affects the staging, with tumor deposits up to 2 cm in diameter representing FIGO 2013 stage IIIB and deposits greater than 2 cm in diameter representing FIGO 2013 stage IIIC.

In cases with obvious macroscopic involvement by carcinoma, one to two blocks of tumor deposits are generally regarded as sufficient sampling [9, 10]. However, in cases where tumor is not apparent macroscopically, more generous sampling is required, as tumor can be detected microscopically in a minority of cases. The exact number of blocks that is required in macroscopically negative cases is not clear and it should be emphasized that detailed macroscopic examination is of prime importance in this situation rather than reliance on microscopic detection. A recommendation of at least four to six blocks in macroscopically negative omentectomy specimens has been suggested as standard practice by the Royal College of Pathologists (London, UK) [1]. Usubutun et al. have recommended three to five blocks in macroscopically negative omentum specimens for patients with high-grade ovarian carcinomas based on their retrospective and prospective study [9]. However, Usubutun et al. admit that further work is required to investigate how many blocks are necessary for low-grade ovarian carcinomas and borderline ovarian neoplasms. More extensive omental sampling is advisable for borderline tumors particularly if there is no macroscopic disease within the omentum. We recommend ten to twelve blocks in the cases with a serous borderline tumor of the ovary with an exophytic capsular component. Furthermore, when noninvasive implants are identified in the omentum associated with a borderline ovarian neoplasm, additional sampling is recommended

as invasive and noninvasive implants can coexist [1]. Additionally, more extensive sampling is advocated for immature teratoma, including when tumor is grossly apparent, as identification of immature teratoma implants will dramatically alter the prognosis and treatment. The American College of Pathologists recommend five to ten blocks to sample macroscopically negative omentum in cases of ovarian serous borderline tumor, serous carcinoma, or immature teratoma, although the authors admit that there is no general consensus on this [11]. In addition to the above, it should also be considered that embedding all omental tissue in macroscopically negative specimens, with the aid of large-sized blocks is standard practice for some pathologists.

Finally, examination of the omentum in cases of post-chemotherapy patients requires random selection of firm areas. These may represent residual tumor or areas of fibrosis, inflammation, and calcification with or without tumor.

Lymph Nodes

Pelvic and/or para-aortic lymph nodes may be received as part of a staging procedure for primary ovarian neoplasms. These are submitted in separate specimen containers and labelled with their site of origin. All the fatty tissue from each specimen site should be measured and the number of lymph nodes retrieved recorded. All lymph node tissue should be sampled with one lymph node placed per cassette. These lymph nodes if enlarged will need slicing transversely (in the short axis) and placing in one cassette. If the lymph node is enlarged such that it cannot be embedded in one cassette, it should be placed in several cassettes making careful note in the block key of how many cassettes that the enlarged node was divided into. Small lymph nodes may be placed in their entirety or bisected if considered appropriate.

Fatty tissue that on palpation feels "knobbly" or finely nodular may contain small lymph nodes, which the authors recommend embedding in several cassettes labelled as such. If an enlarged lymph node is obviously involved by tumor macroscopically, then a representative block should be taken and there is no need to embed the entire lymph node.

Peritoneal Biopsies

Peritoneal biopsies should be submitted in separate specimen containers and labelled as to their site of origin. These biopsies should be measured, submitted in their entirety, and examined at multiple levels if local protocol indicates.

Appendix

An appendix may be received with an ovarian mass specimen in the case of mucinous neoplasms where the possibility of primary appendiceal origin and secondary ovarian involvement with or without pseudomyxoma peritonei should be considered. The appendix and mesoappendix (and any other additional specimens) should be carefully inspected prior to sectioning to look for evidence of mucin or tumor on the serosal surface. It is very important to inspect the appendix for any evidence of rupture. The appendix should then be serially sectioned to look for evidence of tumor in the wall of the appendix and any evidence of mucin within the lumen (Fig. 20.9). In view of the relatively small size of the appendix and the diagnostic challenges of assessing mucinous lesions in the appendix, embedding all tissue is advisable to help confirm the cause of the mucin. A key issue with appendix specimens is to have a good block key. The appendix resection margin must be identifiable in the sections with the aid of this block key, as the status of the appendiceal resection margin may be critical in the clinical decision as to whether a right hemicolectomy is required. If the resection margin cannot be identified in the sections, it may not be possible to assess completeness of excision of an appendiceal neoplasm, thus making management decisions very difficult. Many pathologists take the resection margin block as a transverse section, in which case embedding this slice resection margin down, and indicating this on the block key is advised. Ink can be placed on this slice at

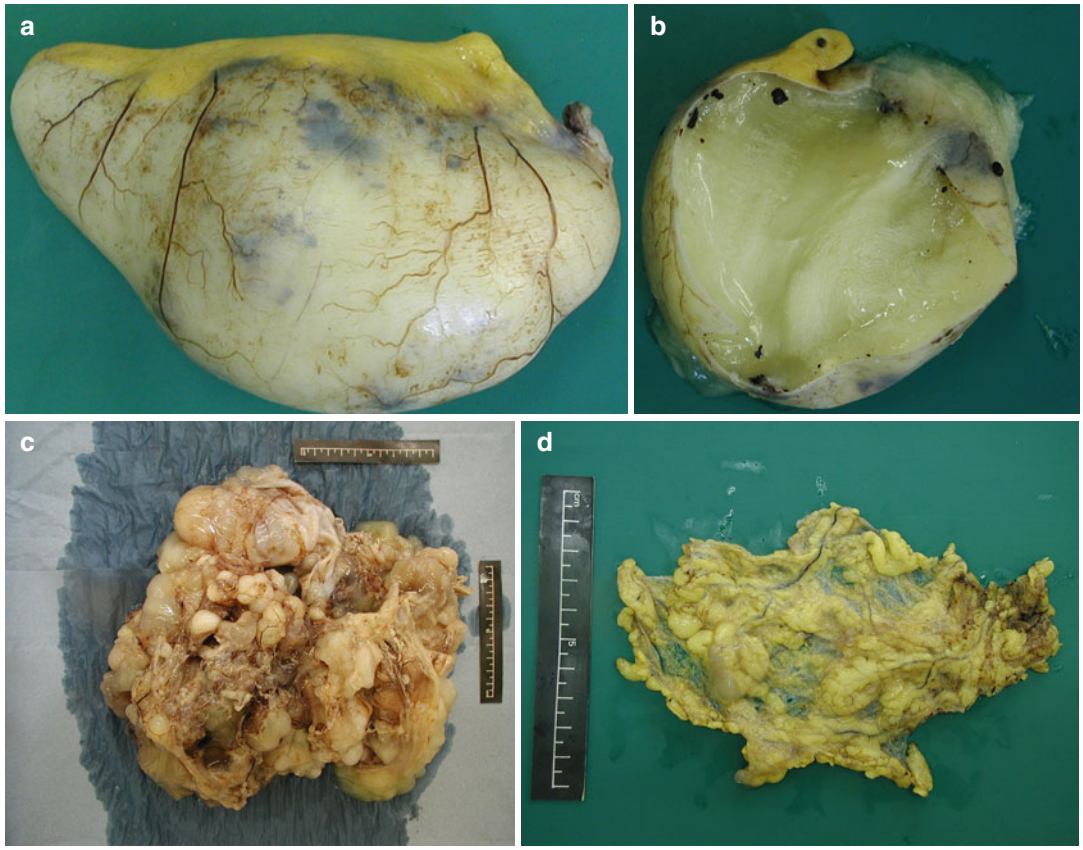


Fig. 20.9 This 10-cm-long appendix (**a**), containing a low-grade appendiceal mucinous neoplasm (LAMN), is dilated up to 5.5 cm in transverse diameter and contains thick mucin within the lumen (**b**). (**c**) A 23-cm-diameter multicystic ovarian tumor containing mucinous material within the cysts, from a patient with pseudomyxoma peritonei and an appendiceal mucinous neoplasm which was the suggested primary origin of the tumor. (**d**) Mucin involving an omentectomy specimen in a patient with pseudomyxoma peritonei

cut-up to indicate to the laboratory staff which surface to embed up/down. Alternatively, some pathologists take longitudinal sections of the resection margin by bisecting a length of appendix close to the resection margin. Longitudinal sections of the appendix tip may be helpful and would be a standard practice for most pathologists with transverse sections of the remaining appendix (or intervening appendix if longitudinal resection margin slices are taken). In addition to labelling the resection margin slice in the block key, indicating how each other slice relates to each other, including embedding one transverse slice per cassette may be helpful for any measurements that may be required, in particular, for measuring the distance of the tumor to the resection margin.

Histologically, two appendiceal lesions in particular low-grade appendiceal mucinous neoplasm (LAMN) and mucinous adenocarcinoma should be looked for carefully [12]. Diverticula can occur in the appendix associated with a mucocele or a LAMN. If a diverticulum is identified in an appendix, it is advisable to embed the entire appendix to confirm the cause of the diverticulum and to exclude an LAMN, adenocarcinoma, or another tumor.

Small Intestine and Large Intestine

Segments of small and large intestines are usually taken because of intestinal obstruction due to extrinsic involvement by tumor or inadvertent

rupture/tearing perioperatively, particularly when there are extensive adhesions. It is important to measure the segment of intestine and to examine the wall and the serosa, including for mucosal lesions. Sampling any serosal tumor deposits and if no abnormalities are noted within the mucosa, then appropriate random blocks of the intestine wall including mucosa should be taken along with sampling of the resection margins.

Spleen

The specimen should be measured and weighed according to local protocol. Blocks representing tumor should be sampled.

Interval Debulking Surgery (IDS) Specimen Cut-Up

Interval debulking surgery (IDS) for advanced ovarian cancer is usually performed after three cycles of chemotherapy have been delivered in a neoadjuvant setting. This is the method of choice when optimum surgical debulking is unlikely. (Please see Chap. 3 for further details regarding surgery.)

Staging is not an issue in this situation as imaging confirms advanced disease. Except in exceptional circumstances, there should always be a tissue diagnosis prior to surgery based on an image-directed core biopsy which may be an omental biopsy or a transvaginal biopsy of a pelvic mass. This is usually performed under either ultrasound or CT guidance. (Please see Chap. 6.) The histopathology in the vast majority of cases is that of high-grade serous carcinoma.

So, what is the purpose of the post-chemotherapy IDS pathological examination? Firstly, to confirm the presence of a primary tumor of gynecological origin, the morphology of which may have been modified by the chemotherapeutic agent [13–15]. Another component may sometimes be identified which was not apparent on the core biopsy such as a sarcomatous component. The tumor could therefore be a carcinosarcoma. Judicious sampling is therefore required. A couple of blocks from each ovary and a couple from the omentum should suffice with regard to sampling the tumor.

There may have been a significant response to the chemotherapy administered, such that little viable tumor remains. The medical oncologist would be interested to know if there is extensive tumor regression with fat necrosis, fibrosis, and inflammation and little residual tumor.

This is also an opportunity for the pathologist to establish if the tumor is genuinely ovarian in origin or tubal in origin depending on the distribution of the disease. Examination of the Fallopian tube may be quite rewarding in these cases when most of the tumor may be localized within the Fallopian tube with adjacent STIC (serous tubal intraepithelial carcinoma) which is known to "survive" the insults of chemotherapy.

Examination of the uterus is mandatory as there may be a coexistent endometrial neoplasm which has eluded the radiologist. Routine blocks of the endometrium should be taken with additional blocks taken as required, should any abnormality become apparent on histological examination. The myometrium should be examined in the usual way, and serosal blocks may be helpful as they would identify tumor on the serosal surface within adhesions.

The cervix should be examined and routine blocks taken to exclude any intraepithelial neoplasia. If any abnormal findings are identified on histological examination, then further blocks may be taken at a later stage. Lymph nodes and peritoneal biopsies may be sampled as above.

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Appendix A: Staging of Ovarian, Fallopian Tube, and Primary Peritoneal Carcinoma

Introduction

Cancer staging is vital for correct patient management, is based on the biology of the individual tumor type, and is determined by internationally agreed protocols which enable comparison and sharing of experience. The major staging body for gynecological cancers is FIGO, and the UICC has broadly developed parallel and concordant systems of TNM staging. The FIGO staging for ovarian cancer has been revised in 2013 (Table A1). The previous staging system was developed in 1988, and two major changes led to recognition of the need to change the existing system: firstly, the knowledge that ovarian cancers are not a single disease with varying morphology but fall into five separate discrete major disease categories and, secondly, insight into the origins of the most common of the subtypes, high-grade serous carcinoma (HGSC). These factors are considered below.

Histological Types of Ovarian Carcinoma

The majority (90 %) of ovarian malignancy is of epithelial origin, i.e., carcinoma, with the remaining 10 % being predominantly of sex cord-stromal or germ cell origin. It is now clear that there are five major subtypes of ovarian carcinoma that differ in their origins, morphology, immunohistochemical staining characteristics, and molecular genetics. These tumor subtypes differ in their behavior and response to treatment. The correct determination of tumor subtype is therefore of as much significance as disease stage, if not of greater significance, a factor even more relevant in the evolving era of personalized medicine and targeted treatment. The histotype of ovarian cancer is therefore included in the 2013 staging system as it is recognized to be fundamental to successful treatment. The five major histotypes of ovarian carcinoma are listed below:

- High-grade serous carcinoma (HGSC)
- Clear cell carcinoma (CCC)
- Endometrioid carcinoma (EC)
- Low-grade serous carcinoma (LGSC)
- Mucinous carcinoma (MC)

Pathogenesis of High-Grade Serous Carcinoma

The second significant discovery influencing the current approach to staging was the recognition in 2001 that a high percentage of HGSC arise in the fimbrial end of the fallopian tube. This discovery was made in risk reducing salpingo-oophorectomy specimens (RRSO) in BRCA1 mutation carriers which were found to harbor serous tubal intraepithelial carcinoma (STIC). Since then STIC has been found in significant numbers of sporadic "ovarian" and "primary peritoneal" carcinomas. Although there is compelling evidence that the majority of HGSC arise from the fallopian tube through studies on RRSO specimens, it is also possible that in some cases STIC represents secondary involvement or evidence of multicentric tumor origin. These

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Table A1 FIGO staging to be used from January 2014: for ovary, fallopian tube, and peritoneum [4]

Existing FIGO stage group	2013 description
Stage 1	*Stage 1*: *Tumor confined to ovaries or fallopian tube*(*s*)
IA	IA: Tumor limited to 1 ovary (capsule intact) or fallopian tube; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings
IB	IB: Tumor limited to both ovaries (capsules intact) or fallopian tubes; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings
IC	(*Stage 1C*) *Tumor limited to 1 or both ovaries or fallopian tubes*, *with any of the following*: IC1: Surgical spill IC2: Capsule ruptured before surgery or tumor on ovarian or fallopian tube surface IC3: Malignant cells in the ascites or peritoneal washings
Stage 2	(*Stage 2*) *Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension* (*below pelvic brim*) *or primary peritoneal cancer*
IIA	IIA: Extension and/or implants on uterus and/or fallopian tubes and/or ovaries
IIB	IIB: Extension to other pelvic intraperitoneal tissues
Stage 3	*Stage 3*: *Tumor involves 1 or both ovaries or fallopian tubes*, *or primary peritoneal cancer*, *with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes*
IIIA	IIIA1: Positive retroperitoneal lymph nodes only (cytologically or histologically proven) IIIA1(i): Metastasis up to 10 mm in greatest dimension IIIA1(ii) Metastasis more than 10 mm in greatest dimension IIIA2: Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes
IIIB	IIIB: Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes
IIIC	IIIC: Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumor to capsule of liver and spleen without parenchymal involvement of either organ)
Stage 4	*Stage 4*: *Distant metastasis excluding peritoneal metastases*
IV	IVA: Pleural effusion with positive cytology IVB: Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

The stage values shown in bold on the left hand column correspond to the 2013 stage values shown in the right hand column and should be used until the options have been updated and aligned to the 2013 classification

alternative possibilities cannot be ruled out at present. It is also relevant that some cases of ovarian and primary peritoneal carcinoma do not show STIC lesions despite complete examination of the fallopian tube using a standard protocol.

While the putative precursor of HGSC is easily identified in RRSO specimens, HGSCs generally present at high stage, at which point any precursor lesions are largely obscured. It is however clear that HGSC, whether presenting as "tubal," "ovarian," or "primary peritoneal" carcinoma based on criteria formerly used to assign primary site, represents a single disease with the majority of cases probably arising in the fallopian tube, even if this is not demonstrable pathologically at the time of presentation. Furthermore HGSC, which accounts for the majority of "ovarian" carcinomas and also the majority of fatal cases, shows identical clinical behavior, including progression patterns and responses to chemotherapy, irrespective of apparent site of origin. For these reasons the FIGO 2013 staging system has been unified for primaries of each of these three sites. Proposals to unify HGSC of ovarian, tubal, and peritoneal origins as "pelvic serous carcinoma" were rejected to avoid confusion by introducing new and ambiguous terminology. As part of staging, however, the primary site for each

case needs to be designated as ovarian, tubal, or primary peritoneal. It is recognized that this may not be possible in some cases and these should be categorized as undesignated.

The remaining minority of ovarian carcinoma subtypes, namely, clear cell, endometrioid, low-grade serous, and mucinous carcinomas, have distinct precursors and origins. The 2013 FIGO staging system is applicable to these subtypes.

Determining the Site of Origin of High-Grade Serous Carcinoma

Complete Sampling of the Fallopian Tube

A fundamental step in pathological evaluation of cases of ovarian, tubal, and primary peritoneal carcinoma has emerged from the aforementioned developments, which is detailed examination of the fimbrial end of the fallopian tube. The recommended protocol, designated SEE-FIM, enables examination of the fimbrial end for the presence of STIC, as detailed in Chap. 20, Specimen Cutup. This step is essential for correct staging as the changes are not visible to the naked eye and there is a potential to under-stage disease if these are missed. In addition, although currently there is no universal agreement on how the presence of STIC should influence assignment of site of origin, recording this finding systematically will provide evidence to inform future guidance.

Problems in Assigning Site of Origin

STIC lesions are found in 60 % of BRCA1-positive high-grade serous carcinomas and an undetermined percentage of sporadic ovarian carcinomas. In the remainder the relative proportions of true tubal and ovarian origin are not known. There is also the possibility that rather than representing a precursor, the presence of STIC and similar lesions represents secondary involvement or even multicentric origin. There is no accepted consensus regarding terminology in these cases with polar views being " when the origin is uncertain, the convention of designating all serous carcinoma as originating in the ovary should not be used" [2] and "the term HGSC of ovary should be kept until the different origins are better understood" [1].

In this scenario there is high potential for categorizing identical cases differently. For example, high-grade serous carcinoma with low-volume ovarian involvement, widespread peritoneal spread, and STIC in the fallopian tube could potentially be categorized as being of fallopian tube, ovary, primary peritoneal, or undesignated origin by different pathologists. Similarly it is also possible for an ovarian mass with complete obscurement of the fallopian tube where demonstration of a precursor lesion is not possible and an identical case of advanced ovarian carcinoma in the presence of demonstrable STIC to be categorized differently as ovarian/undetermined and fallopian tubal, respectively, if STIC is taken to be proof of origin whenever it is found. Another difficult scenario is assigning origin to a large ovarian mass which obscures the ipsilateral fallopian tube in the presence of STIC on the contralateral fallopian tube. One further confounding factor is the aggressive nature of STIC; it is accepted that this lesion, although intraepithelial, is capable of widespread metastasis and presentation as ovarian or primary peritoneal carcinoma; it therefore should be included in cancer staging as an involved site, irrespective of the absence of invasive disease in the tube. While the new staging system ensures that the patient will be staged and therefore treated and prognosticated identically, there is potential for confusion with regard to assignment of primary site.

Suggestions for Assigning Site of Origin

In the absence of internationally agreed guidelines, it is not possible to give a recommendation for every possible scenario. Overall assignment of site of origin should be based on common sense, experience, and professional judgment but a few broad suggestions are listed below. This is

not an exhaustive list nor intended to be binding, and assignment of origin in an individual case is left to the discretion of the pathologist and the local multidisciplinary team:

1. The fallopian tubes, or at least their fimbrial ends, should be totally sampled in all cases of HGSC by a SEE-FIM-like protocol to avoid missing this important site of disease, which probably represents the precursor lesion and tumor origin in the majority of cases.
2. The presence of STIC, in the absence of invasive disease in the fallopian tube, should be considered as tubal involvement for staging purposes; by the same token, the presence of STIC without invasion or extratubal spread should be staged as FIGO stage IA tubal carcinoma (although these have favorable prognosis, based on limited experience to date) but with an annotation that there is no invasive carcinoma.
3. Cases with only STIC, ovarian surface involvement or parenchymal involvement not exceeding 5 mm, and widespread peritoneal involvement, which would traditionally be categorized as primary peritoneal carcinoma, should be classified as tubal primaries.
4. Cases with invasive HGSC located within the mucosa of the fallopian tube, including its fimbrial end, with or without STIC in any portion of the fallopian tube and with no, minimal, or even substantial ovarian involvement should be categorized as tubal primaries.
5. Cases in which the fallopian tube is not identifiable, having presumably been overgrown by the ipsilateral adnexal mass, or the distal end of the fallopian tube is incorporated into a large tubo-ovarian mass should also, based on current understanding, be diagnosed as tubal primaries. It is emphasized that a careful effort must be made to identify the tube in all cases.
6. Cases with dominant ovarian mass(es) and identifiable fallopian tubes with STIC should be classified as tubal primaries.
7. Cases with dominant ovarian mass(es) and identifiable fallopian tubes without STIC should be classified as ovarian primaries.
8. Cases should be categorized as primary peritoneal carcinoma by the conventional criteria below and only after complete examination of the fallopian tubes (including the non-fimbrial portions) have excluded the presence of STIC or a small HGSC:
   - Both ovaries must be normal in size or enlarged by a benign process.
   - The involvement in the extraovarian sites must be greater than the involvement on the surface of either ovary.
   - The ovarian tumor involvement must be nonexistent, be confined to the ovarian surface without stromal invasion, or involve the cortical stroma with tumor size less than 5×5 mm.
9. All cases classified as "undesignated" for FIGO staging purposes should be further described as "tubo-ovarian" or "tubal/ovarian" to distinguish them from serous carcinoma originating in the uterus. Using our suggestions, these should represent a small proportion of HGSC.
10. Cases with unilateral or bilateral HGSC in the ovary and/or STIC or HGSC in the tube but with an endometrial serous intraepithelial or invasive carcinoma should be carefully evaluated for an endometrial versus a tubo-ovarian primary (WT1 may be of value); a majority of such cases will represent adnexal metastases from an endometrial serous carcinoma.

The 2013 FIGO (and TNM) Staging of Ovarian, Fallopian Tube, and Primary Peritoneal Carcinoma Including Summary of Changes Since the 1988 FIGO Staging

The 2013 FIGO Staging of Ovarian, Fallopian Tube, and Primary Peritoneal Carcinoma

The staging system is given below. This has been approved by the American Joint Commission on Cancer and the International Union Against

Cancer, and the equivalent TNM stages are given alongside.

The following are implicit in the 2013 staging system as indicated in the notes at the end:

- The tumor type should be designated as HGSC, EC, CCC, MC, and LGSC; other or cannot be classified; and malignant germ cell tumors and potentially malignant sex cord-stromal tumors.
- The primary site (i.e., ovary, fallopian tube, or peritoneum) should be designated where possible (see "Suggestions for assigning site of origin"). In some cases, it might not be possible to delineate the primary site clearly; such cases should be listed as "undesignated."

Stage I: Tumor Confined to Ovaries or Fallopian Tube(s)

T1-N0-M0

IA: Tumor limited to 1 ovary (capsule intact) or fallopian tube; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings

T1a-N0-M0

IB: Tumor limited to both ovaries (capsules intact) or fallopian tubes; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings

T1b-N0-M0

IC: Tumor limited to 1 or both ovaries or fallopian tubes, with any of the following:

IC1: Surgical spill

T1c1-N0-M0

IC2: Capsule ruptured before surgery or tumor on ovarian or fallopian tube surface

T1c2-N0-M0

IC3: Malignant cells in the ascites or peritoneal washings

T1c3-N0-M0

Comment

Stage I ovarian or fallopian tube cancer is confined to the ovaries or the fallopian tubes and peritoneal fluid/washings. Tumor rupture or surface involvement by tumor cells warrants a stage of IC. It is not possible to have stage I peritoneal cancer.

Stage II: Tumor Involves 1 or Both Ovaries or Fallopian Tubes with Pelvic Extension (Below Pelvic Brim) or Primary Peritoneal Cancer

T2-N0-M0

IIA: Extension and/or implants on uterus and/or fallopian tubes and/or ovaries

T2a-N0-M0

IIB: Extension to other pelvic intraperitoneal tissues

T2b-N0-M0

Comment

Stage II ovarian cancer is still difficult to define. It comprises a small and heterogeneous group making up less than 10 % of ovarian cancers. It is defined as extension or metastasis to extraovarian/extratubal pelvic organs and may include curable tumors that have directly extended to adjacent organs but have not yet metastasized, as well as tumors that have seeded the pelvic peritoneum by metastasis and, therefore, have a poor prognosis. Of note, the sigmoid colon is within the pelvis, and therefore sigmoid involvement only is considered stage II. The Committee felt that subdividing this small category further into IIB1 and IIB2 (i.e., microscopic and macroscopic pelvic peritoneal metastases) was not based on evidence/biology. All stage II disease is treated with adjuvant chemotherapy, so subclassification is not essential. Also, the old substage IIC (i.e., IIA or IIB but with tumor on surface, capsule ruptured, or ascites or positive peritoneal washing) was considered redundant and eliminated.

Stage III: Tumor Involves 1 or Both Ovaries or Fallopian Tubes, or Primary Peritoneal Cancer, with Cytologically or Histologically Confirmed Spread to the Peritoneum Outside the Pelvis and/or Metastasis to the Retroperitoneal Lymph Nodes

T1/T2-N1-M0

IIIA1: Positive retroperitoneal lymph nodes only (cytologically or histologically proven):

IIIA1(i) Metastasis up to 10 mm in greatest dimension

IIIA1(ii) Metastasis more than 10 mm in greatest dimension

IIIA2: Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes

T3a2-N0/N1-M0

IIIB: Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes

T3b-N0/N1-M0

IIIC: Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumor to capsule of liver and spleen without parenchymal involvement of either organ)

T3c-N0/N1-M0

Comment

Most ovarian cancers are HGSCs that usually present in stage III, with the vast majority (84 %) stage IIIC. These tumors characteristically spread along peritoneal surfaces involving both pelvic and abdominal peritoneum including the omentum, surfaces of the small and large bowel, mesentery, paracolic gutters, diaphragm, and peritoneal surfaces of the liver and spleen. A finding of ascites occurs in two-thirds of cases. Lymph node metastases are found in the majority of patients who undergo node sampling or dissection and in up to 78 % of advanced stage patients. Approximately 9 % of patients with tumors that otherwise appear to be stage I have lymph node metastases; the corresponding figures for stages II, III, and IV are 36, 55, and 88 %, respectively. Rarely, inguinal or supraclavicular (stage IV) node metastases will be the presenting manifestation of ovarian carcinoma.

Less than 10 % of ovarian carcinomas extend beyond the pelvis with exclusively retroperitoneal lymph node involvement. Evidence in the literature indicates that these cases have a better prognosis than that of tumors with abdominal peritoneal involvement. The new staging includes a revision of stage III patients and assignment to stage IIIA1 based on spread to the retroperitoneal lymph nodes without intraperitoneal dissemination. Stage IIIA1 is further subdivided into IIIA1(i) (metastasis ≤10 mm in greatest dimension) and IIIA1(ii) (metastasis >10 mm in greatest dimension), even if there are no retrospective data supporting quantification of the size of metastasis in IIIA1. Involvement of retroperitoneal lymph nodes must be proven cytologically or histologically.

Stage IV: Distant Metastasis Excluding Peritoneal Metastases

Stage IVA: Pleural effusion with positive cytology

Stage IVB: Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)

Any T, any N, M1

Comment

Stage IV is defined as distant metastasis and includes patients with parenchymal liver/splenic metastases and extra-abdominal metastases; 12–21 % of patients present with stage IV disease. Extension of tumor from omentum to spleen or liver (stage IIIC) should be differentiated from isolated parenchymal metastases (stage IVB).

Notes

- The primary site (i.e., ovary, fallopian tube, or peritoneum) should be designated where possible. In some cases, it might not be possible to delineate the primary site clearly; such cases should be listed as "undesignated."
- The histologic type should be recorded.
- The staging includes a revision of stage III patients; assignment to stage IIIA1 is based on spread to the retroperitoneal lymph nodes without intraperitoneal dissemination because an analysis of these patients indicates that their survival is significantly better than that of patients with intraperitoneal dissemination.
- Involvement of retroperitoneal lymph nodes must be proven cytologically or histologically.
- Extension of tumor from omentum to spleen or liver (stage IIIC) should be differentiated from isolated parenchymal metastases (stage IVB).

Summary of Changes from the 1988 FIGO Staging

The two staging systems and changes are summarized in Table A2. There is no longer a separate staging system for tubal and primary peritoneal carcinoma. Tumor type is to be included in the stage. The primary site should be designated as ovary, fallopian tube, or primary peritoneal wherever possible or as "undesignated" if not (see "Suggestions for assigning site of origin").

Table A2 Comparison of 1988 and 2013 FIGO staging systems for ovarian cancer

1988 FIGO stage	2013 FIGO stage	Difference
I: Tumor limited to the ovaries	I: Tumor confined to ovaries or fallopian tube(s)	No change; same staging for ovary and fallopian tube
IA: Tumor limited to 1 ovary; capsule intact, no tumor on ovarian surface, no malignant cells in ascites or peritoneal washings	IA: Tumor limited to 1 ovary (capsule intact) or fallopian tube; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings	No change; same staging for ovary and fallopian tube
IB: Tumor limited to both ovaries; capsule intact, no tumor on ovarian surface, no malignant cells in ascites or peritoneal washings	IB: Tumor limited to both ovaries (capsules intact) or fallopian tubes; no tumor on ovarian or fallopian tube surface; no malignant cells in the ascites or peritoneal washings	No change; same staging for ovary and fallopian tube
IC: Tumor limited to 1 or both ovaries with any of the following: capsule ruptured, tumor on ovarian surface, malignant cells in ascites or peritoneal washings	IC: Tumor limited to 1 or both ovaries or fallopian tubes, with any of the following: IC1: Surgical spill IC2: Capsule ruptured before surgery or tumor on ovarian or fallopian tube surface IC3: Malignant cells in the ascites or peritoneal washings	Change: Separation into substages depending on presence of surgical spill or capsule rupture prior to surgery or surface involvement or malignant cells in ascites/peritoneal washings
II: Tumor involves 1 or both ovaries with pelvic extension	II: Tumor involves 1 or both ovaries or fallopian tubes with pelvic extension (below pelvic brim) or primary peritoneal cancer	No change; same staging for ovary, fallopian tube, and primary peritoneal cancer
IIa: Extension and/or implants on uterus and/or tube(s); no malignant cells in ascites or peritoneal washings	IIA: Extension and/or implants on uterus and/or fallopian tubes and/or ovaries	No change; same staging for ovary, fallopian tube, and primary peritoneal cancer
IIB: Extension to other pelvic tissues; no malignant cells in ascites or peritoneal washings	IIB: Extension to other pelvic intraperitoneal tissues	No change; same staging for ovary, fallopian tube, and primary peritoneal cancer
IIC: Pelvic extension IIA or IIB with malignant cells in ascites or peritoneal washings		Change: There is no stage IIC in the 2013 staging system
III: Tumor involves 1 or both ovaries with microscopically confirmed peritoneal metastasis outside the pelvis and/or regional lymph node metastasis	III: Tumor involves 1 or both ovaries or fallopian tubes, or primary peritoneal cancer, with cytologically or histologically confirmed spread to the peritoneum outside the pelvis and/or metastasis to the retroperitoneal lymph nodes	No change; same staging for ovary, fallopian tube, and primary peritoneal cancer
IIIA: Microscopic peritoneal metastasis beyond pelvis	IIIA1: Positive retroperitoneal lymph nodes only (cytologically or histologically proven): IIIA1(i) Metastasis up to 10 mm in greatest dimension IIIA1(ii) Metastasis more than 10 mm in greatest dimension IIIA2: Microscopic extrapelvic (above the pelvic brim) peritoneal involvement with or without positive retroperitoneal lymph nodes	Change (1): Regional nodal metastasis was classified as stage IIIC in the 1988 staging system Change (2): Stage IIIA is subdivided into: IIIA1: Retroperitoneal lymph node involvement, substaged by dimension of metastatic deposits IIIA2: Microscopic extrapelvic peritoneal involvement +/− nodal spread

(continued)

Table A2 (continued)

1988 FIGO stage	2013 FIGO stage	Difference
IIIB: Macroscopic peritoneal metastasis beyond pelvis 2 cm or less in greatest diameter	IIIB: Macroscopic peritoneal metastasis beyond the pelvis up to 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes	No change in dimensions of peritoneal deposits but irrespective of nodal involvement
IIIC: Peritoneal metastasis beyond pelvis more than 2 cm in greatest diameter and/or regional lymph node metastasis	IIIC: Macroscopic peritoneal metastasis beyond the pelvis more than 2 cm in greatest dimension, with or without metastasis to the retroperitoneal lymph nodes (includes extension of tumor to capsule of liver and spleen without parenchymal involvement of either organ)	Change: This stage is now defined solely by dimensions of peritoneal tumor deposits, irrespective of retroperitoneal nodal spread. Nodal involvement alone, without peritoneal spread, is now classified as stage IIIA
IV: Distant metastasis (excludes peritoneal metastasis)	IV: Distant metastasis excluding peritoneal metastases	Change: Separation into substages IVA and IVB
Note: Liver capsule metastasis is stage III, liver parenchymal metastasis is stage IV. Pleural effusion must have positive cytology for stage IV	Stage IVA: Pleural effusion with positive cytology Stage IVB: Parenchymal metastases and metastases to extra-abdominal organs (including inguinal lymph nodes and lymph nodes outside of the abdominal cavity)	

Controversies, Problematic Areas in the New Staging System with Recommendations

Many continuing controversies are discussed alongside the new staging system, and it is acknowledged that these cannot be resolved until further data emerge. Recommendations on the use of this staging system and for future research have been made. These are listed and briefly discussed below.

- It is acknowledged that bilateral involvement (stage IB) could represent independent contralateral primary tumor or implants/metastases with potentially different prognostic implications.
- Assessment of surface involvement requires careful gross examination. Surface involvement of the ovary or fallopian tube should be considered present only when tumor cells are exposed to the peritoneal cavity, characterized by exophytic papillary tumor on the surface of the ovary or fallopian tube or on the outer surface of a cystic neoplasm replacing these organs; rarely, a smooth ovarian tumor surface will be shown to have an exposed layer of neoplastic epithelium on microscopic examination.
- Involvement of fimbrial end of the tube by STIC alone does not constitute surface involvement; such lesions should be staged as IA as they have been demonstrated to have a good prognosis.
- Invasive carcinoma at the fimbrial end of the tube should be taken as constituting surface involvement as this portion of the tube is exposed to the peritoneal surface.
- Distinction between rupture prior to surgery and during surgery may not be possible without access to the surgical notes or after discussion at the multidisciplinary team meeting.
- Dense adhesions often cause rupture during surgery and could represent involvement of pelvic tissues by tumor. At present tumors are not upstaged based on dense adhesions as the available data are inconclusive. Staging in the presence of adhesions depends on tumor rupture or microscopic evidence of involvement of extraovarian pelvic tissues.
- Histologic grade has been shown in several studies to influence prognosis of stage I tumors. The tumor grade is now implicit in the tumor type for HGSC, CCC, and LGSC and currently most grade 3 ECs are considered to be HGSC. EC and MC should continue to be graded and distinguished from metastases

from the gastrointestinal tract or female genital tract.
- It is not established whether rupture during surgery worsen prognosis in the absence of excrescences, ascites, or positive washings.
- Data from several studies suggest that stage I CCC is more frequently stage IC compared with other cell types, possibly because of an increased risk of rupture and/or surface involvement due to coexisting endometriosis.
- It is unclear whether positive washings are worse than capsule rupture as a prognostic factor. Separation into separate categories of stage IC should help to resolve such issues in the future.
- It may not be biologically justified to separate the pelvic from the extrapelvic peritoneum. Some investigators have the view that the peritoneum is an anatomic unit and that pelvic and extrapelvic involvement are prognostically similar and therefore that all peritoneal involvement should be regarded as stage III. This view was not supported unanimously and stages IIA and IIB remain.
- It is possible that some carcinomas that have extended beyond the pelvis with exclusively retroperitoneal lymph node involvement (stage IIIA1) represent independent LGSCs arising in retroperitoneal lymph nodes from endosalpingiosis. Serous borderline tumors and LGSCs may develop in retroperitoneal and cervical lymph nodes from endosalpingiosis, often in association with serous borderline tumors of the ovary and with favorable prognosis.
- The new stage IIIA1 is limited to involvement of the retroperitoneal lymph nodes below the diaphragm. It was suggested that upward nodal involvement (e.g., mediastinal nodes) should be included, but, for now, the Committee felt that the stated limitation was appropriate.
- Cytological confirmation of involved nodes or peritoneal disease would be insufficient for substaging into IIIA1 and IIIA2.
- Regarding the amount of peritoneal involvement, it was maintained that stage III tumors should be divided into microscopic and macroscopic peritoneal spread, and for the latter the measurement (in centimeters) of the largest dimension of a single nodule should be given. Further, distinction should be made between single small lesions within the omentum (<2 cm) and diffuse peritoneal disease including the upper abdomen and diaphragm.
- Transmural bowel infiltration with mucosal involvement and umbilical deposits (both currently IVB) are specifically mentioned. Some consider that involvement of the umbilicus should be IIIC rather than IV as it represents peritoneal extension into the urachal remnant. Similarly, isolated parenchymal liver metastasis and splenic parenchymal metastasis are susceptible to cytoreductive surgery and, according to some investigators, should be IIIC. None of these were adopted by the FIGO Committee, i.e., transmural bowel infiltration, umbilical deposits, and parenchymal metastases in the liver and spleen are all considered stage IVB.
- The results for splenectomy for isolated metastases are superior to those of partial hepatectomy. In the future, isolated splenic metastasis may be considered stage IIIC rather than stage IV, whereas parenchymal liver metastasis would remain stage IVB.
- It is unclear whether positive lymph nodes above the renal vessels should be considered stage III or IV.

Recommendations for Stage I
- Histologic type, which in most cases includes grade, should be recorded.
- All individual subsets of stage IC disease should be recorded.
- Dense adhesions with histologically proven tumor cells justify upgrading to stage II.
- If rupture is noted, peritoneal washing and cytology study are indicated.

Recommendations for Stage II
- To separate direct extension from metastases
- To compare outcome of stage II and early stage III cases

Recommendations for Stage III
- To classify IIIA1 cases histologically
- To compare outcome of stage IIIA1(i) and IIIA1(ii) cases
- To compare outcome of stage IIIA1 and IIIA2 cases

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6. National Cancer Intelligence Network: cancer outcomes and services dataset: http://www.ncin.org.uk/collecting_and_using_data/data_collection/cosd

Appendix B: Datasets and Information About Gynecological Cancer Web Sites

We have not provided a dataset for completion as these are presently being updated by the Royal College of Pathologists and are being written by the International Collaboration on Cancer Reporting (ICCR).

We recommend that these Web sites are consulted for the most recent dataset, and for further helpful information further links have been provided.

Royal College of Pathologists link to cancer dataset publications: http://www.rcpath.org/publications-media/publications/datasets/datasets-TP.htm

ICCR dataset link: http://www.rcpa.edu.au/Library/Practising-Pathology/ICCR/Cancer-Datasets

This is a link to the United Kingdom Cancer Profiles: http://www.ncin.org.uk/cancer_type_and_topic_specific_work/cancer_type_specific_work/gynaecological_cancer/gynaecological_cancer_hub/profiles

This is a link to the cancer services and outcomes datasets: http://www.ncin.org.uk/collecting_and_using_data/data_collection/cosd

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