Gynecological Cancers: Pathology and Cytological Methods for Diagnosis of Gynecological Cancers

3

Nalini Gupta and Arvind Rajwanshi

Abstract

Gynecological cancers represent cancers arising from the female genital tract. The most common cancer in female genital tract is carcinoma cervix. Carcinoma cervix is preceded by precancerous lesions, which can be detected by screening tests such as cervical Pap smear and HPV detection. Endometrial carcinoma is usually detected on endometrial curettings or biopsy, as endometrial aspiration cytology is not reliable. Cervical smear may occasionally represent cells arising from endometrial carcinoma. Majority of ovarian tumors are epithelial tumors, papillary serous carcinoma being the most common. Although FNAC in ovarian neoplasms is not a preferred technique, the same may be performed in young females as initial work-up, in advanced ovarian malignancies, and in the follow-up of patients after chemotherapy or radiotherapy. This chapter includes pathology of gynecological cancers and cytological evaluation of the same such as cervical Pap test for cervical carcinomas and fine needle aspiration cytology mainly for ovarian tumors.

Abbreviations

AGCT	Adult granulosa cell tumor
AIS	Adenocarcinoma in situ
CCC	Clear cell carcinoma
CEA	Carcinoembryonic antigen
CIN	Cervical intraepithelial neoplasia
CIS	Carcinoma in-situ
CK	Cytokeratin
ESS	Endometrial stromal sarcoma
FDA	Food and drug administration
FNAC	Fine needle aspiration cytology
H&E	Hematoxylin and Eosin stain
HPV	Human papilloma virus
HSIL	High grade squamous intraepithelial lesions
JGCT	Juvenile granulosa cell tumor
LBC	Liquid based cytology
LMS	Leiomyosarcoma
LSIL	Low-grade squamous intraepithelial lesions

N. Gupta • A. Rajwanshi (✉)
Department of Cytology and Gynaecological Pathology, Postgraduate Institute of Medical Education and Research, Chandigarh 160 012, India
e-mail: rajwanshiarvind@gmail.com

M.K. Shetty (ed.), *Breast and Gynecological Cancers: An Integrated Approach for Screening and Early Diagnosis in Developing Countries*, DOI 10.1007/978-1-4614-1876-4_3,

MGG	May Grünwald Geimsa stain
MMMT	Malignant mixed müllerian tumor
Pap	Papanicolaou stain
PAS	Periodic acid-Schiff's stain
PCR	Polymerase chain reaction
SCC	Squamous cell carcinoma
SIL	Squamous intraepithelial lesions
STIC	Serous tubal intra-epithelial carcinoma
TBS	The Bethesda System
VIA	Visual inspection using acetic acid
VILI	Visual inspection using Lugol's iodine

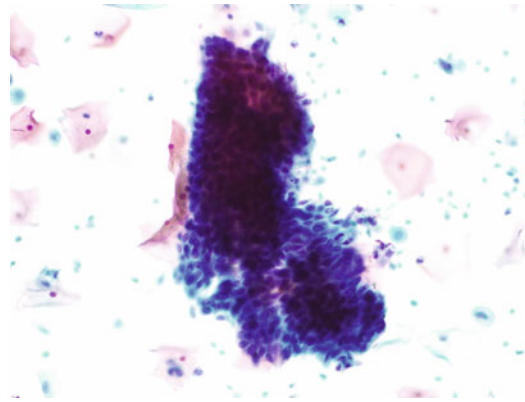

Fig. 3.1 SurePath™ (BD Worldwide, Franklin Lakes, NJ) LBC cervical sample showing a hyperchromatic crowded cell group (HCCG) representing severe dysplasia/HSIL (Pap x100X)

Introduction

Gynecological cancers represent cancers arising from female genital tract, which consists of the uterus and cervix, vagina, vulva, bilateral fallopian tubes, and ovaries. Among the gynecological cancers, cervical carcinoma is the most common cancer and the second most common cancer among women worldwide, with an estimated 529,000 new cases and 274,000 deaths annually as per GLOBOCAN 2008 [1]. About 80% of the cervical cancer cases occur in developing countries. Endometrial cancer is the second most common gynecological cancer, followed by ovarian carcinoma. In general, epithelial tumors are the more common; however, sarcomas can occur in cervix, uterus, vagina, and vulva. Rarely melanoma, lymphoma, and metastatic tumors can involve female genital tract. In addition to epithelial tumors, germ cell tumors and sex cord stromal tumors are known to arise from the ovaries. Peritoneal fluid cytology is done for staging of gynecological cancers, especially for ovarian and endometrial cancers.

Carcinoma of the Uterine Cervix

Cervical cancer is the most or second most common cancer among women in developing countries. The age-standardized rate for incidence is 15.2/100,000 women and for mortality is 7.8/100,000 women. The incidence of cervical cancer in India varies from 16.3 to 30.6 per lakh [2]. Cervical cancer has marked differences in incidence according to demographic variables (age, social class, marital status, ethnicity, religion, and occupation). The majority of cervical cancer is squamous cell carcinoma (SCC), and human papilloma virus (HPV) has been strongly associated with cervical carcinoma, especially HPV 16 and HPV 18. Cervical cancer is unique as it is preceded by precancerous lesions, which can be detected by screening methods and treated.

Cervical Intraepithelial/Precancerous Lesions

Persistent HPV infection of cervical squamous epithelium can lead to squamous intraepithelial lesions (SIL), which can be low-grade (LSIL) and high-grade SIL. According to The Bethesda System (TBS) 2001, LSIL includes koilocytosis and CIN1, and HSIL includes moderate dysplasia (CIN2), severe dysplasia (CIN3) (Figs. 3.1 and 3.2), and carcinoma in situ (CIS). About 60% of CIN1 lesions regress, 30% persist, 10% progress to CIN3, and 1% to invasive SCC. CIN2 lesions regress in 40% of the cases, 40% persist, 20% progress to CIN3, and 5% progress to SCC (Figs. 3.3 and 3.4). The likelihood of CIN3 regressing is 33%, and the likelihood of progressing to SCC is more than 12% [3, 4].

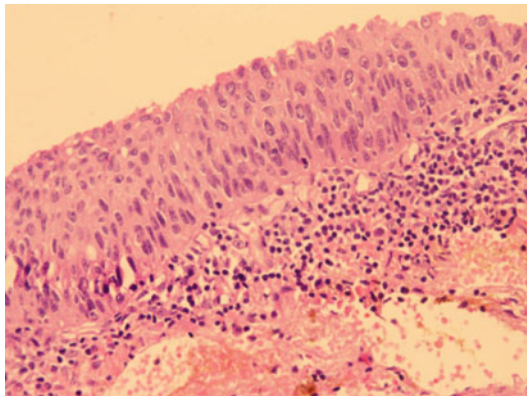

Fig. 3.2 Cervical biopsy showing CIN 3 (cervical intraepithelial neoplasia) changes (H&E x40X)

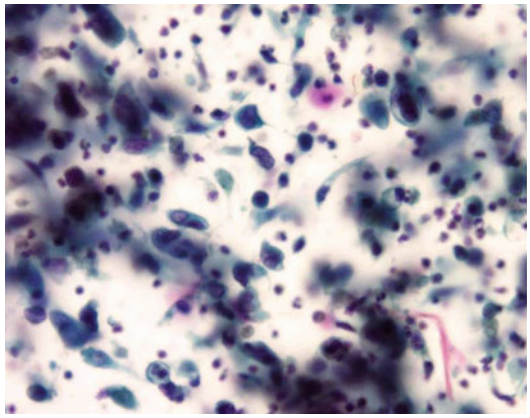

Fig. 3.3 SurePath™ (BD Worldwide, Franklin Lakes, NJ) LBC cervical sample showing malignant squamoid cells of varying sizes and shapes in squamous cell carcinoma cervix (Pap x100X)

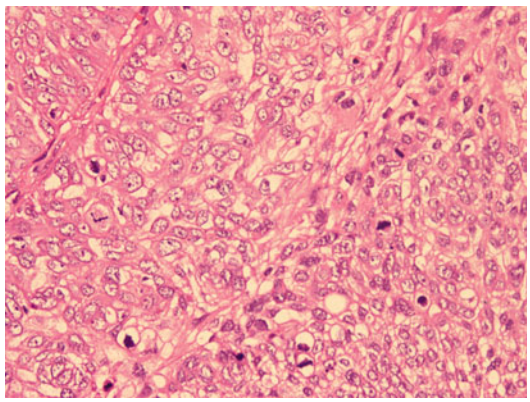

Fig. 3.4 Cervical biopsy showing squamous cell carcinoma cervix (H&E x40X)

Adenocarcinoma in situ (AIS) is a preinvasive glandular lesion of uterine cervix. Nearly two-thirds of cases of AIS have associated preinvasive squamous lesions or invasive SCC [5, 6], and risk factors for AIS are similar to that of preinvasive squamous lesions [7].

Screening for Cervical Precancerous Lesions

The objective of cervical cancer screening programs is to reduce the incidence of the disease and mortality from the disease by identifying women in precancerous stage or early invasive cancers and treating these women appropriately. The conventional screening modality for cervical pre-cancer is the cytological Pap smear test. Cervical Pap cytology by conventional method has worked efficiently for the last 50 years, with more than 80% potential decrease in mortality from cervical cancer in developed countries. This test was first introduced by Papanicolaou and Babes in 1920, and, since then, despite the proven effectiveness of conventional Pap smear, the accuracy of conventional smear has been questioned. Liquid-based cytology (LBC) and HPV testing are another two relatively new technologies in the field of cervical cancer screening. LBC technique was introduced in the mid-1990s to improve the performance of Pap test. LBC improves specimen adequacy and reduces screening time as compared to conventional cytology. Other methods for cervical screening include unaided visual inspection of cervix, visual inspection using acetic acid (VIA), visual inspection using Lugol's iodine (VILI), and colposcopic examination. Computer-assisted cytological interpretation of cervical smears, use of physical real time devices, and detection of molecular surrogate markers of cervical cancer—such as ProEx™ C, $p16^{INK4A}$, pRb, p27, p53, MCM5 and CDC6, Ki-67, etc.—have also been evaluated [8, 9]. Dual immunostaining for $p16^{INK4a}$ and Ki-67 on cervical smears has been evaluated, and kits are commercially available for the same [9, 10]. Among these, cervical cytology is still the mainstay of cervical cancer prevention programs.

Cervical Cytology

Two techniques available for cervical cytology are (1) conventional method and (2) liquid-based cervical cytology. Conventional Pap smear involves collection of exfoliated cells from the cervix, especially the transformation zone, by a spatula/collection device, and the sample is smeared on the glass slide and stained with Papanicolaou stain. These can result in false negative diagnoses either due to sampling or screening errors. Sampling errors can occur when abnormal cells are not scraped off, abnormal cells stick on the wooden spatula resulting in cell loss, or the cells transferred to the slide may not be representative of an abnormality. Screening error can occur if the smears are too thick with numerous polymorphs, mucus, or blood obscuring the abnormal cells.

Liquid-based cytology includes a number of different LBC techniques, such as ThinPrep™ (Hologic Inc., Bedford, MA), SurePath™ (BD Worldwide, Franklin Lakes, NJ), Cytoeasy, Cytoscreen, PapSpin, etc. The US Food and Drug Administration (FDA) approved two liquid-based tests for cervical screening, namely the ThinPrep™ in 1996 and the SurePath™ in 1999. Pap test by LBC involves the use of Cervex-Brush® (Rovers Medical Devices, Oss, The Netherlands) or a combination of a plastic spatula and endocervical brush to collect cervical sample from transformation zone. The head of the spatula/brush is either kept in the vial containing preservative by detaching the head of the brush (SurePath™ samples) or removed after rinsing the spatula/brush in the preservative vial (ThinPrep™ samples). In ThinPrep™, clumps of cells and mucus are broken by mechanical agitation, and then this solution is filtered to allow inflammatory cells and red blood cells to pass through and epithelial cells are retained. These epithelial cells are then passed on to the glass slide and stained. In SurePath™ LBC method, the cellular clumps are broken, and the cells are separated with the help of density gradient centrifugation. The epithelial cell pellet is resuspended and transferred to the glass slide. The diameter of the circle for ThinPrep™ sample is 22 and 18 mm for SurPath™ sample.

LBC is proposed to have many benefits over conventional cytology such as less number of unsatisfactory/inadequate smears, more representative transfer of cells from collecting device to the slide, the cellular material is evenly distributed on the slide, residual cellular material can be used for HPV testing, reduced screening time, and possibly higher rate of HSIL detection. The reduction of inadequate rate from 9.1% in conventional cytology to 0.9% by using SurePath™ technique has been reported [11]. Diagnostic accuracy of LBC as compared to conventional cytology is a matter of great debate, as several studies have shown increased sensitivity of LBC over conventional method [12–14], whereas others have shown decreased or equal sensitivity and specificity [15, 16].

Human Papilloma Virus Testing

Another test used in screening programs especially to triage women with low-grade SIL lesions is high risk HPV DNA testing. Various techniques for hrHPV detection include immunocytochemical techniques, southern blotting, filter in situ hybridization, Digene Hybrid Capture™ (Digene, Gaithersburg, MD) HCII assay, Polymerase chain reaction (PCR), and genotyping methods [8, 17, 18]. FDA approved Qiagen's (Hilden, Germany) "hybrid-capture" test for detection of HPV infection in 2003. Polymerase chain reaction has also been used for viral load determination [19].

Pathology of Invasive Cervical Carcinoma

Grossly, these tumors may be endophytic and diffusely infiltrative or exophytic polypoidal tumors with a cauliflower-like appearance. Hysterectomy is the treatment of choice only in early stage tumors and, in such cases, the extent of upper and lateral involvement, vaginal cuff involvement, and depth of cervical stromal infiltration by the tumor are important to evaluate. On microscopy, the most common carcinoma is SCC (Fig. 3.4), followed by an endocervical adenocarcinoma

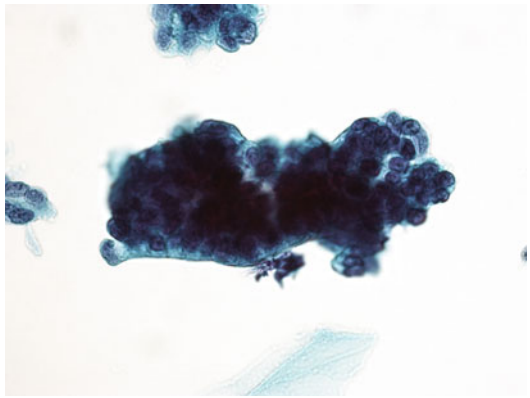

Fig. 3.5 SurePath™ (BD Worldwide, Franklin Lakes, NJ) LBC cervical sample showing a cluster of atypical cells representing an endocervical adenocarcinoma (Pap x100X)

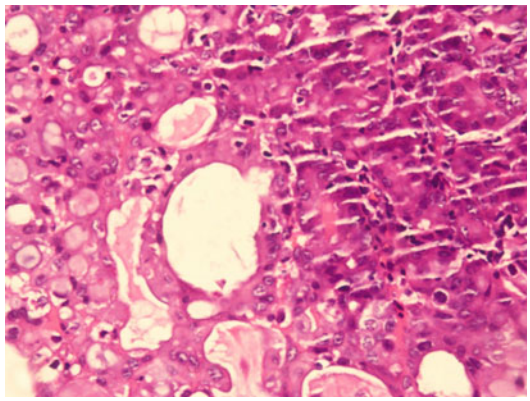

Fig. 3.6 Cervical biopsy showing an endocervical adenocarcinoma (H&E x40X)

(Figs. 3.5 and 3.6). Other uncommon types include adenosquamous, verrucous, warty, glassy cell, clear cell adenosquamous, neuroendocrine, adenoid-cystic, Mucoepidermoid, and small cell carcinoma. Adenocarcinoma of the cervix can be minimal deviation adenocarcinoma, mucinous adenocarcinoma, endometrioid adenocarcinoma, and clear cell adenocarcinoma.

Secondary Tumors

Cervix can be involved by direct extension from endometrial, rectal, or urinary bladder tumors. Metastases to the cervix are rare, the most common sites being the gastrointestinal tract, the ovary, the breast, lung, kidney, and, rarely, melanoma. The tumor cells from cervical metastatic lesions may get sampled in Pap smears and rarely are the first indication of the tumor [20].

Malignant Tumors of the Uterus

Malignant tumors of the uterus include endometrial carcinoma, endometrial stromal sarcoma, and malignant mixed müllerian tumor (MMMT). Malignant tumor arising from the smooth muscle of the myometrium is leiomyosarcoma.

Endometrial Carcinoma

Endometrial carcinoma is divided into two forms: type I tumors (endometrioid carcinoma) account for 70–80% of cases and are estrogen related, whereas the type II tumors (papillary serous or clear cell tumors) account for about 20% of cases, are unrelated to estrogen stimulation, and have an aggressive clinical behavior [21]. This dualistic model of endometrial tumorigenesis was proposed by Bokhman in 1983. Obesity, nulliparity, hypertension, and diabetes are risk factors for type I carcinomas, and these often develop in a background of endometrial hyperplasia. Other predisposing factors include dysfunctional uterine bleeding, estrogen replacement therapy, polycystic ovarian disease, and tamoxifen use. Most serous (type II) cancers have p53 mutations [22], and endometrioid (type I) adenocarcinoma demonstrates larger numbers of genetic changes including microsatellite instability (MSI), or specific mutation of PTEN, K-ras, and β-catenin genes [23, 24].

Cytologic screening of endometrial cancer is possible by direct endometrial sampling or cervical Pap sample. However, these are difficult to interpret and are only of moderate reliability. Endometrial biopsy or curettage is usually performed to detect any endometrial pathology. 1–11% of the cases with the presence of endometrial cells on Pap smear may be associated with endometrial adenocarcinoma (Fig. 3.7) [25, 26].

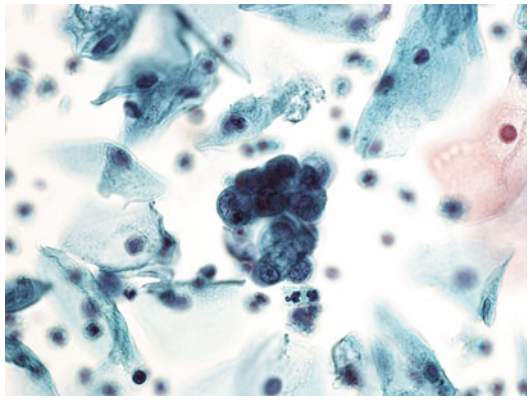

Fig. 3.7 SurePath™ (BD Worldwide, Franklin Lakes, NJ) LBC cervical sample showing a three dimensional cluster of atypical cells representing endometrioid type of endometrial adenocarcinoma (Pap x100X)

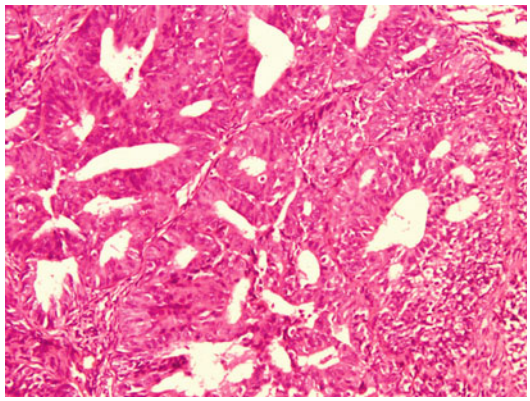

Fig. 3.8 Endometrial curettings showing a well-differentiated endometrioid adenocarcinoma (H&E x40X)

Type I: Endometrioid Adenocarcinoma

Type I: endometrioid adenocarcinoma accounts for almost 70–80% of all endometrial carcinomas. The majority of females are postmenopausal, and only 1–8% of endometrial carcinomas occur in women under 40 years of age [27]. The most common clinical presentation is abnormal vaginal bleeding. The gross appearance in this tumor may vary from shaggy endometrial surface to exophytic polypoidal growth arising from endometrium and infiltrating the myometrium. On microscopy, these tumors comprise of complex glandular pattern (Fig. 3.8) and can have solid to villoglandular appearance. These are divided into three grades using both architectural and nuclear criteria. Squamous differentiation may be a prominent feature. It is important to evaluate myometrial invasion, lower uterine segment, and endocervical involvement by tumor. It may be difficult to distinguish a well-differentiated endometrioid adenocarcinoma from atypical complex endometrial hyperplasia in curettage samples.

Prognostic factors include both uterine and extra-uterine factors. Uterine factors include histologic type and grade, depth of myometrial invasion, cervical involvement, lympho-vascular space invasion, presence of complex atypical hyperplasia, and hormone receptor status. Extra-uterine factors include adnexal involvement, intra-peritoneal metastasis, positive peritoneal cytology, and pelvic and para-aortic lymph node metastasis.

Type II: Serous Carcinoma and Clear Cell Carcinoma

Type II: serous carcinoma and clear cell carcinoma present as highly aggressive tumors that occur in older women as compared to type I tumors. Papillary serous carcinoma is characterized by predominant complex papillary pattern. The lining tumor cells are usually polygonal with eosinophilic to clear cytoplasm, and these cells exhibit marked nuclear atypia (Figs. 3.9 and 3.10). Serous carcinoma is characterized by the discordance between its architectural pattern and nuclear morphology. These tumors usually show strong nuclear immunopositivity for p53.

Clear cell carcinoma usually exhibits solid, tubular, papillary, or cystic patterns. The tumor cells are usually clear to eosinophilic and show prominent "hobnail" pattern. Nuclear atypia may be marked, and PAS positive, diastase-resistant intracellular as well as extracellular hyaline globules may be noted. This tumor is usually a high grade, deeply invasive tumor, and the prognosis is poor.

Malignant Mixed Müllerian Tumor (Carcinosarcoma)

MMMT accounts for less than 5% of malignant uterine tumors. It is a biphasic tumor composed

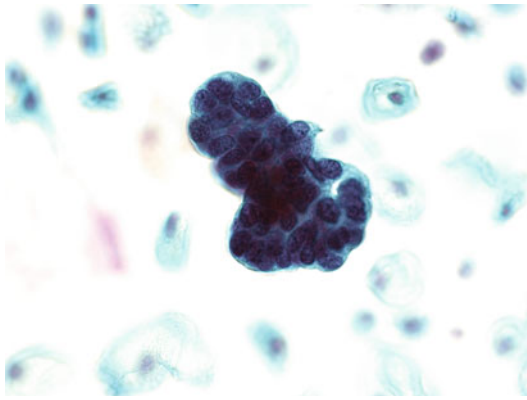

Fig. 3.9 SurePath™ (BD Worldwide, Franklin Lakes, NJ) LBC cervical sample showing a papillary cluster of atypical cells representing papillary serous type of endometrial adenocarcinoma (Pap x100X)

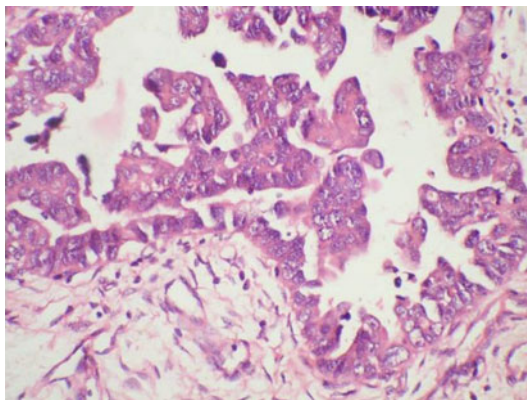

Fig. 3.10 Endometrial curettings showing a papillary serous type of endometrial adenocarcinoma (H&E x40X)

of malignant epithelial as well as sarcomatous components. Majority of the women are postmenopausal and present with postmenopausal bleeding. Grossly, MMMTs are usually polypoidal tumors that fill whole of the uterine cavity, often protruding through the cervical os. Microscopically, these are divided into homologous type and heterologous type. The most common type of epithelial component is endometrioid adenocarcinoma. Serous, clear cell, mucinous, squamous carcinoma can occur. Mesenchymal component is usually endometrial stromal sarcoma or fibrosarcoma and rarely leiomyosarcoma. When heterologous component is present, rhabdomyosarcoma and chondrosarcoma are the most common types identified. These tumors may metastasize to pelvic and paraaortic lymph nodes, pelvis, vagina, peritoneal surfaces of abdomen, and lungs.

Endometrial Stromal Sarcoma

Endometrial stromal sarcoma (ESS) is a tumor of endometrial stromal cells that invades the myometrium. ESS is divided into low-grade ESS and high-grade ESS. These occur in the fourth and fifth decades of life, and usually patients are premenopausal. Abnormal vaginal bleeding, cyclical menorrhagia, and abdominal pain are the frequent presenting symptoms. Grossly, the myometrium may be diffusely thickened or a nodular tumor may be evident in the endometrial cavity. The tumor permeates the myometrium and may be seen as poorly demarcated yellowish tiny nodules or cords in dilated channels. On microscopy, most ESS are of low grade type with relatively uniform appearing small oval tumor cells, which resemble stromal cells. Numerous small blood vessels and arterioles may be noted throughout the tumor resembling spiral arterioles. The individual tumor cells are enveloped by reticulin fibers. The nests, trabeculae, and cords of tumor cells invade the myometrium by invasion of lympho-vascular channels, which is a characteristic feature of this tumor. Areas of hyaline fibrosis may be noted. The tumor cells in ESS stain positive for CD10 antigen and are negative for Caldesmon. Smooth muscle actin and desmin are also usually negative, and therefore these stains may be of help in differentiating ESS from leiomyosarcoma. This tumor rarely is subjected to fine needle aspiration cytology (FNAC). On FNAC, the tumor cells are usually present in loose clusters and singly. The nuclei are plump, oval, and moderately pleomorphic. The chromatin is usually bland, and one or two small nucleoli may be noted [28]. High-grade ESS is defined as a sarcoma without specific features or heterologous elements but with an infiltrative pattern suggestive of an origin from endometrial stromal cells [29].

Müllerian Adenosarcoma

Müllerian adenosarcoma is a biphasic tumor composed of benign epithelial component and a sarcomatous stroma. Grossly, it is usually a polypoid tumor growing in the endometrial cavity. On microscopy, the fibrous/stromal papillary structures are lined by bland-appearing epithelium, and tubular and cleft-like structures are seen within the stroma. The mesenchymal component can be homologous or heterologous type. Müllerian adenosarcoma with a sarcomatous overgrowth (MASO) is an aggressive variant of this tumor.

Leiomyosarcoma

Leiomyosarcoma (LMS) accounts for about 1.3% of the uterine malignancies and about one-third of uterine sarcomas. It usually affects women in their sixth decade of life. The common symptoms are abnormal vaginal bleeding, lower abdominal pain/distension, or awareness of abdominal mass. Grossly, majority of LMS are solitary intramural masses. The cut surface is soft or fleshy, and areas of hemorrhage and necrosis may be seen. Irregular infiltrative margins may or may not be identifiable grossly. On microscopy, the tumor is highly cellular composed of fascicles of cigar-shaped to spindle cells showing nuclear atypia. Tumor cell necrosis is usually prominent but need not be present. Mitoses are numerous (>10/10 high power fields). These tumors should be distinguished from atypical and mitotically active leiomyoma. Epithelioid and myxoid variants of LMS are known. Myxoid variant is of particular importance as the mitotic index and cellularity may not be high, but tumor size of more than 5 cm is significant. These tumors are rarely subjected to FNAC or any other cytological techniques for diagnosis.

Tumors of Vagina and Vulva

SCC accounts for more than 80% of all malignancies (Fig. 3.11). Other malignancies include clear cell adenocarcinoma, embryonal rhabdomyosarcoma (sarcoma botryoid), malignant melanoma, lymphoma, leiomyosarcoma, and aggressive angiomyxoma. These tumors, especially carcinomas and melanomas, can also be sampled rarely in cervico-vaginal Pap smears.

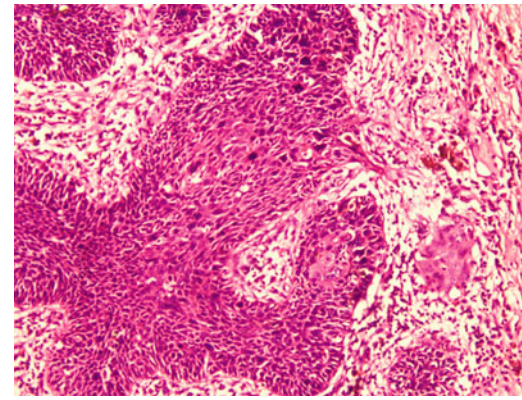

Fig. 3.11 Vulval biopsy showing a superficially invasive squamous cell carcinoma (H&E x40X)

Vulval Paget's Disease

Vulval Paget's disease presents as an erythematous or eczematous lesion which usually involves hair-bearing skin. A biopsy shows large pale cells with finely granular to vacuolated cytoplasm. These cells form nests or a layer along the basement membrane and are positive for mucin and MUC1and MUC5AC. The incidence of an underlying carcinoma varies from 0 to 30% in vulval Paget's disease. This can also be detected by scrape cytology and smears show pale, large vacuolated cells demonstrating nuclear pleomorphism with or without associated inflammation.

Ovarian Tumors

Ovarian cancers are the fifth most common cause of mortality from all cancers in women in the developed world [30]. The proportion of ovarian cancer varies from 1.7 to 8.7% of all female cancers in various urban and rural population-based registries operating under the network of the National Cancer Registry program (NCRP) of

Indian Council Medical Research (ICMR) [31]. Ovarian cancer rates increase with rising age. Hereditary ovarian cancer occurring in approximately 10% cases is mainly due to mutations in BRCA1 or BRCA2 tumor suppressor genes. An effective screening test is not possible for the general population because ovarian cancer is uncommon and the ovaries are not easy to access. High risk females can be screened by serial ultrasonography with/without serum CA125 levels. Ovarian cancer can spread locally to contra-lateral ovary, peritoneum, and para-aortic and pelvic lymph nodes. Patient may present with ascites. Despite controversial views regarding FNAC in ovarian lesions, it can be useful in initial workups of primary ovarian neoplasms, biopsy of superficial masses in patients with known prior disease, and follow-up of patients undergoing chemotherapy/radiotherapy [32].

Classification of Ovarian Tumors

Ovarian tumors are classified based on the cell of origin:

1. Surface epithelial tumors account for about 90–95% of ovarian malignancies. These are further divided into serous, mucinous, endometrioid, transitional, mixed tumors, and malignant mixed Müllerian tumor (MMMT).
2. Germ cell tumors account for about 15–20% of all ovarian neoplasms and are divided into:
   (a) Dysgerminoma
   (b) Teratoma: mature and immature
   (c) Endodermal sinus tumor (Yolk sac tumor)
   (d) Embryonal carcinoma
   (e) Choriocarcinoma
   (f) Mixed germ cell tumor
3. Sex cord-stromal tumors: account for about 5–10% of all ovarian neoplasms and are categorized into:
   (a) Granulosa cell tumor: adult and Juvenile type
   (b) Fibroma-thecoma
   (c) Sertoli leydig cell tumor
   (d) Leydig cell tumor
   (e) Others
4. Metastatic tumors account for about 5% of ovarian malignancies, and usually arise from breast, colon, endometrium, stomach, gall bladder, and cervical cancers.
5. Other rare tumors arise from ovarian soft tissue, etc.

Ovarian Surface Epithelial Tumors

Pathogenesis of Ovarian Surface Epithelial Tumors

According to the most recent findings, the majority of "ovarian" carcinomas arise outside the ovary from the fimbrial end of the fallopian tube. A dualistic model has been proposed which divided ovarian carcinomas into type I and type II [33]. Type I tumors are generally low-grade indolent tumors and are usually confined to the ovary. These exhibit a shared lineage between benign cystic tumors and the corresponding carcinomas, often through an intermediate (borderline tumor) step, supporting the morphologic continuum of tumor progression. These are characterized by specific mutations including KRAS, BRAF, ERBB2, CTNNB1, and PTEN. Type II tumors are aggressive and present in advanced stage. These tumors are genetically unstable and show TP53 mutations in more than 80% cases [33].

Serous Carcinoma/Papillary Serous Cystadenocarcinoma

Serous carcinoma is the most common type of ovarian cancer accounting for approximately 50% of all malignant ovarian tumors. The peak age is in the fifth and sixth decades, and approximately two-thirds of cases involve both ovaries. Serum CA125 levels are elevated in about 90% of the cases, and about 70–84% cases present in advanced stage with disseminated disease involving abdominal and pelvic cavities [34].

Grossly, these tumors can have wide variation in size and are typically multilocular and cystic with soft friable papillae filling the cysts containing serous or bloody fluid. There are solid areas which can have variegated appearance due to hemorrhage and necrosis. On microscopy, serous carcinoma typically displays a complex papillary pattern with solid areas. The papillae are lined by cuboidal to low columnar epithelium which

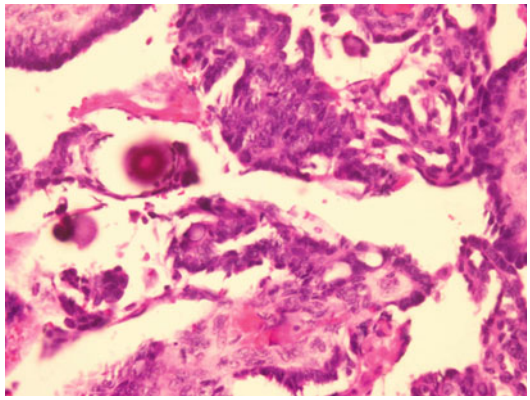

Fig. 3.12 Papillary serous adenocarcinoma of the ovary with psammoma bodies (H&E x40X)

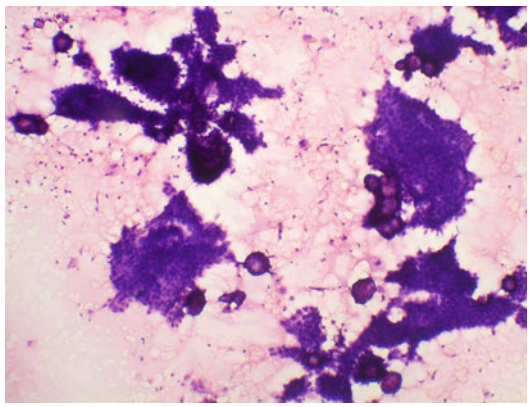

Fig. 3.13 FNAC of papillary serous adenocarcinoma of the ovary with psammoma bodies (H&E x20X)

shows nuclear pleomorphism, atypia, and pseudostratification. Psammoma bodies are seen in about 25% cases (Fig. 3.12). Serous tumors are positive for epithelial membrane antigen, Cytokeratin 7, and CA125. CK20 is negative in a majority of serous ovarian carcinomas.

FNAC is usually performed in advanced stage tumors for tissue diagnosis. Usually the smears are highly cellular and show papillary clusters and dispersed population of malignant cells exhibiting nuclear atypia (Fig. 3.13). Psammoma bodies may or may not be seen. The differential diagnosis on cytology includes reactive mesothelial proliferation, endometrioid carcinoma, and metastatic carcinoma.

Mucinous Carcinoma/Mucinous Cystadenocarcinoma

These comprise approximately 11% of all ovarian carcinomas and are bilateral in about 10–12% cases. These tumors generally present as a large abdomino-pelvic mass due to their large size. About 63% of patients have FIGO stage I disease. Ascites may occur, but pseudomyxoma peritonei is associated with mucinous tumors of appendix and not with ovarian mucinous tumors. Grossly, these are usually large, multiloculated cystic tumors with solid areas and intracystic nodules. The cysts contain thick viscous mucinous material. On microscopy, the cystic areas are lined by tall columnar mucinous lining epithelium which can be endocervical or intestinal type. Papillae project into cystic spaces and the spaces contain eosinophilic mucinous material. Stromal invasion, architectural complexity, and nuclear stratification and atypia are the characteristic features. Pools of mucin may be seen in adjacent ovarian stroma leading to inflammatory reaction. Mucinous carcinomas are positive for carcinoembryonic antigen (CEA), CK7 and about 70% cases are positive for CK20. Majority of these tumors are CDX2 negative. The main differential diagnosis includes metastatic colorectal carcinoma, which is CK7+ and CK20+ and also CDX2 positive.

FNAC usually yields thick highly viscous mucinous material. Single cells, irregular clusters, and syncytial groups of atypical cells are seen in smears. The cells usually show nuclear atypia, which is not as prominent as seen in serous carcinomas.

Endometrioid Adenocarcinoma

Endometrioid adenocarcinoma comprises of approximately 17.5% of ovarian carcinomas. Co-existent endometriosis may be demonstrated in 15–20% cases. Synchronous endometrioid tumors of the uterus may be seen in about 10–30% cases. Grossly, the tumors have a smooth outer surface, and the cut section is usually solid and cystic with cysts containing soft friable masses and hemorrhagic fluid. On microscopy, the tumor is typically characterized by a complex glandular proliferation with back-to-back arrangement and

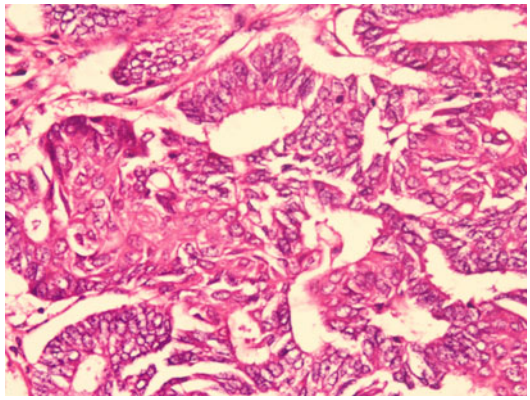

Fig. 3.14 Endometrioid adenocarcinoma of the ovary with focal squamous differentiation (H&E x40X)

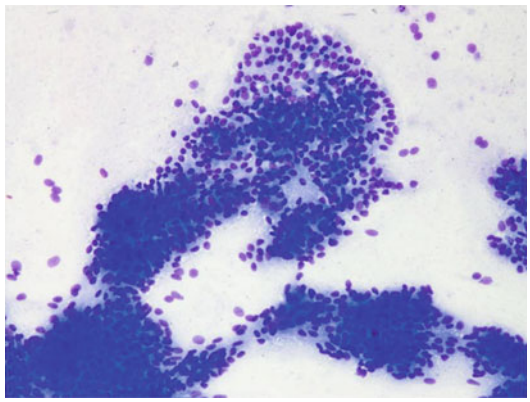

Fig. 3.15 FNAC of endometrioid adenocarcinoma of the ovary (MGG x40X)

stromal invasion. Confluent or cribriform proliferation of glands and solid areas may be seen. The cystic spaces are lined by tall columnar epithelium. Squamous differentiation may be noted in up to 50% cases (Fig. 3.14). About one-third of cases may exhibit endometrioid component mixed with other epithelial components such as clear cell or papillary serous component. These tumors are CK7 positive and CK20 negative.

On FNAC, the malignant cells may resemble cells of serous carcinoma. Micro-acini and tall columnar cells with granular cytoplasm favor endometrioid carcinoma (Fig. 3.15).

Clear Cell Carcinoma

Clear cell carcinoma (CCC) comprises about 7.4% of ovarian carcinomas. The average age at the time of diagnosis ranges from 48 to 58 years. This tumor has been associated with paraneoplastic hypercalcemia. Twenty-four to fifty percent of the patients are known to have associated pelvic endometriosis [35]. According to Yoshikawa and colleagues, the order of the prevalence of endometriosis in each histologic type is clear cell (39.2%)>endometrioid (21.2%)>serous (3.3%)>mucinous type (3.0%) [36]. Grossly, the tumor shows honeycomb cut surface or a thick-walled cyst with fleshy nodules. On microscopy, this tumor displays several different patterns such as solid, tubulo-papillary, papillary, and tubule-cystic. The solid pattern has sheets of polyhedral cells having abundant clear cytoplasm, which may exhibit PAS positive hyaline globules (Fig. 3.16). These cells are separated by delicate fibrovascular sepate. The cores of papillae often exhibit prominent hyalinization. The tumor cells are usually large, pleomorphic, and have prominent cell borders and prominent nucleoli. Nuclei may protrude into the cystic lumina giving a "hob-nail" appearance. The main differential diagnosis includes yolk sac tumor and dysgerminoma. IHC panel comprising of CK7, EMA, CD15 (Leu M1), and alpha fetoprotein (αFP) is recommended to differentiate between yolk sac tumor and clear cell carcinoma of the ovary [37]. The tumor cells in CCC are positive for CK7, EMA, and CD15 and negative for αFP.

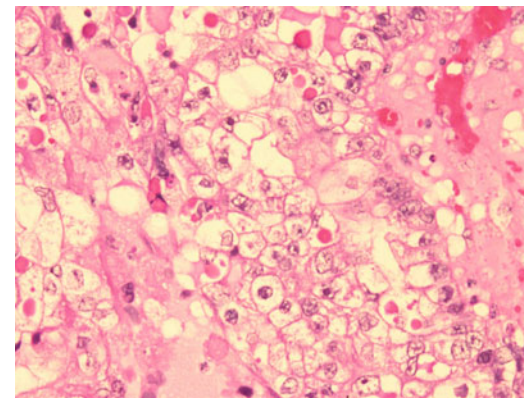

Fig. 3.16 Clear cell carcinoma of the ovary with hyaline globules (H&E x40X)

On FNAC, CCC is characterized by cells with abundant clear to pale, vacuolated cytoplasm

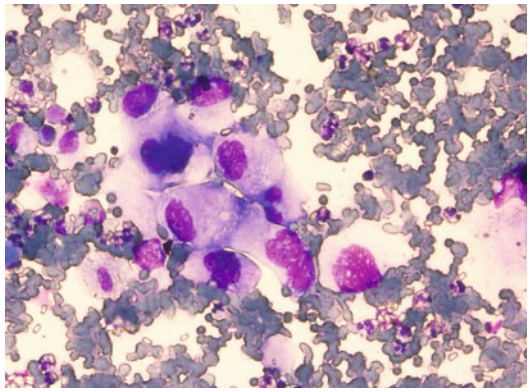

Fig. 3.17 FNAC of clear cell carcinoma of the ovary (MGG x40X)

(Fig. 3.17). The nuclear pleomorphism may be evident with prominent nucleoli. The tumor should be differentiated from metastatic renal cell carcinoma.

Malignant Transitional Cell Tumor

Some investigators have divided malignant transitional cell tumor into malignant Brenner tumor, in which a benign or atypical proliferative Brenner component is identified, and a transitional cell carcinoma, in which these components are not identified. On microscopy, thick, blunt papillary folds with fibrovascular folds are noted, and these are lined by transitional epithelium as seen in urothelial carcinomas.

Small Cell Carcinoma

Small cell carcinoma is a rare, aggressive tumor with a dimorphic population of large and small malignant cells. This tumor is often associated with paraneoplastic hypercalcemia [38].

Malignant Mixed Müllerian Tumor (Carcinosarcoma)

MMMT comprises less than 1% of ovarian neoplasms and resembles its uterine counterpart. It is characterized by an intimate admixture of malignant epithelial and sarcomatous components; these tumors can be of the homologous or heterologous type. Only epithelial or sarcomatous components may be represented on FNAC.

Germ Cell Tumors of the Ovary

Malignant germ cell tumors mainly occur in children and women less than 30 years of age.

Dysgerminoma

Dysgerminoma is the most common malignant germ cell tumor of the ovary and accounts for 1–2% of malignant ovarian tumors. The most common presenting symptom is abdominal mass and pain. It is bilateral in 5–10% cases, and it is usually a common component of mixed germ cell tumor. Grossly, dysgerminoma is usually a firm large solid tumor with smooth nodular external surface. The cut surface is homogenous, lobulated, light tan or whitish, and fleshy. On microscopy, it is composed of well-defined nests, islands, and cords of tumors cells separated by thin fibrous septae infiltrated by lymphocytes. The tumor cells are large, oval to round, uniform cells with well-defined cell borders and clear to finely granular cytoplasm containing glycogen. The nuclei show one or more prominent nucleoli. PAS stain helps to demonstrate the intracytoplasmic glycogen. About 5% cases can have HCG positive syncytiotrophoblastic cells, which may lead to elevated serum HCG levels. Granulomatous inflammation may be noted. Sometimes, there is marked anaplasia of the tumor cells, and the tumor is designated as anaplastic dysgerminoma. Dysgerminoma is placental specific alkaline phosphatase (PLAP) and CD117 (C-kit).

On FNAC, the smears are usually cellular and show predominantly dispersed population of large round fragile tumor cells with clear to vacuolated cytoplasm, central round nuclei, pale chromatin, and one or more prominent nucleoli. Another characteristic feature is tigroid background of the smear due to the glycogen, which is better appreciable in MGG-stained smears. Background shows lymphocytes and plasma cells. Differential diagnosis on cytology includes poorly differentiated carcinoma, large cell lymphoma, and malignant melanoma.

Immature Teratoma

Immature teratoma is a malignant teratoma comprising of an admixture of mature and immature/

embryonal tissues derived from all the three germ layers: ectoderm, mesoderm, and endoderm. It accounts for less than 1% of teratomas of the ovary, the majority being dermoid cysts and mature cystic teratomas. It occurs predominantly in children and young adults. Patients usually present with abdominal mass and pain. Rarely, patients have acute abdominal symptoms due to torsion or rupture of the tumor. Grossly, it is usually unilateral and can co-exist with a mature teratoma in the contra-lateral ovary. The tumor is large and firm, and the cut surface is both solid and cystic. Solid areas can be fleshy or hard and gritty. The cystic areas may show hair follicles or tooth as seen in mature cystic teratoma. It is important to sample various areas of the tumor in order to demonstrate various components of immature teratoma. On microscopy, in addition to mature component derived from ectodermal, mesodermal, and endodermal layers, immature components are seen. Primitive neuroepithelium and neuroblasts are seen along with immature embryonal stroma and cartilage. Immature teratoma should be graded, as the prognosis depends upon the grade and stage of the tumor. The most widely used grading system divides immature teratoma into three grades, from grade 1 for a tumor showing limited amount of immature tissue (not >1 low power field/slide) to grade 3 for a tumor with abundant immature tissue (≥4 low power fields/slide). Immature teratoma may have areas of hyalinization and necrosis, leftover predominant mature elements, or maturation of immature components after chemotherapy.

Carcinoid Tumor

Carcinoid Tumor is an uncommon tumor and is usually a part of a teratoma. Ovarian carcinoids are found mainly in peri- or postmenopausal women. About one-third are associated with carcinoid syndrome. On gross examination, the tumor has a smooth surface, and the cut surface is solid, tan to yellow, and homogeneous. Microscopically, the morphology is as seen in carcinoid tumors elsewhere with organoid trabecular, insular, or solid patterns. The tumor cells are uniform with fine speckled chromatin and inconspicuous nucleoli. Strümal carcinoid shows a combination of carcinoid tumor with Strüma ovarii.

Yolk Sac Tumor (Endodermal Sinus Tumor)

Yolk Sac Tumor is a neoplasm of children, adolescents, and young adults. Elevated serum αFP (alpha fetoprotein) is almost always demonstrable in these tumors. Grossly, the tumors are unilateral and large, and on the cut surface they are solid and are cystic with areas of hemorrhage and necrosis. On microscopy, the majority of the tumors show a combination of different patterns, most common being the reticular or microcystic pattern. Other patterns include festoon or pseudo-papillary pattern; alveolar–glandular, myxomatous, macrocystic, hepatic, polyvesicular vitelline pattern; and solid pattern. Schiller–Duval bodies are a characteristic feature. PAS positive diastase-resistant intracytoplasmic hyaline globules are seen.

On FNAC, the smears show irregular, large, three-dimensional papillary or ball-like clusters of malignant cells with irregular coarse chromatin and prominent nucleoli. These clusters are usually seen associated with brightly eosinophilic extracellular matrix material. Hyaline globules and Schiller–Duval bodies may be seen. It is important to evaluate if this is a component of mixed germ cell tumor; therefore, FNAC should be attempted from different areas of the tumor.

Embryonal Carcinoma

Embryonal carcinoma is uncommon in the ovary as compared to testis, and it is usually a component of mixed germ cell tumor. Presenting symptoms include abdominal pain and hormone-related symptoms such as menstrual irregularity or precocious pseudopuberty. Serum β-HCG levels are usually elevated along with αFP levels. These tend to be large, solid, and fleshy tumors grossly. On microscopy, the tumor cells are markedly pleomorphic and are arranged in cords, glands, and papillae (Fig. 3.18). Syncytiotrophoblastic cells are usually present along with cellular stromal tissue.

Choriocarcinoma

Pure primary ovarian choriocarcinoma is extremely rare.

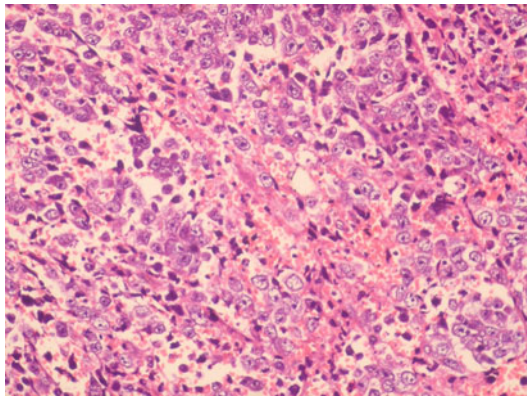

Fig. 3.18 Embryonal carcinoma of the ovary with markedly pleomorphic tumor cells (H&E x40X)

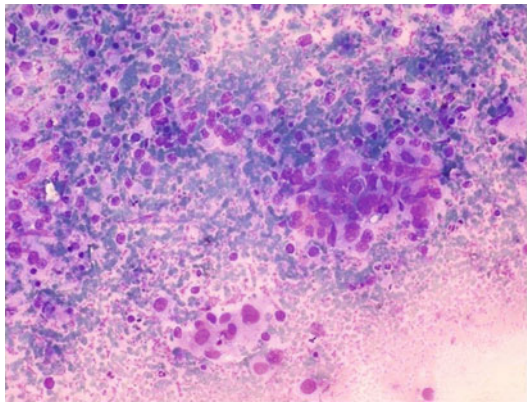

Fig. 3.19 FNAC of mixed germ cell tumor representing embryonal carcinoma component (MGG x40X)

Malignant Mixed Germ Cell Tumor

Malignant mixed germ cell tumors contain a mixture of malignant germ cell elements, the most common being a combination of dysgerminoma and yolk sac tumor (Fig. 3.19). Embryonal carcinoma, choriocarcinoma, and polyembryoma are rare.

Sex Cord-Stromal Tumors

These account for about 5–10% of all ovarian neoplasms.

Granulosa Cell Tumor

Adult Granulosa Cell Tumor

Adult granulosa cell tumor (AGCT) accounts for about 1–2% of all ovarian tumors and more than

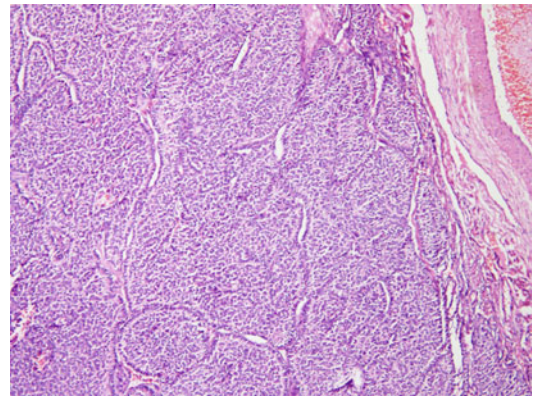

Fig. 3.20 Solid pattern in a case of adult granulosa cell tumor (H&E x40X)

90% of all granulosa cell tumors. AGCT mainly occurs in peri- or postmenopausal women. These are the most common estrogenic ovarian tumors clinically, and about 5–10% patients have endometrial carcinoma. The most common presenting symptom is postmenopausal bleeding. Rarely, these tumors are androgenic or hormonally inactive. Serum inhibin is a useful marker. Grossly, these may vary in size and are encapsulated with a smooth-lobulated outline. The cut surface may be solid or predominantly cystic. On microscopy, the variety of patterns can be seen, such as micro and macrofollicular, trabecular, water-silked, insular, solid- tubular, gyriform, or sarcomatoid (Fig. 3.20). Call–Exner bodies are a characteristic feature, and the tumor cells have angulated nuclei and nuclear grooves resulting in a "coffee-bean" appearance. The presence of theca cells in varying quantities may be seen. FNAC is rarely attempted and may show small sized tumor cells with eosinophilic round bodies (Fig. 3.21).

Juvenile Granulosa Cell Tumor

The majority of juvenile granulosa cell tumor (JGCT) occurs in the first three decades of life. Most common presentation is pseudo-precocity. Grossly, these tumors are similar to AGCT and are usually solid as well as cystic masses. On microscopy, there is a diffuse solid or macrofollicular growth pattern with follicular lumina filled with eosinophilic or basophilic secretions. The tumor cells generally have round, hyperchromatic

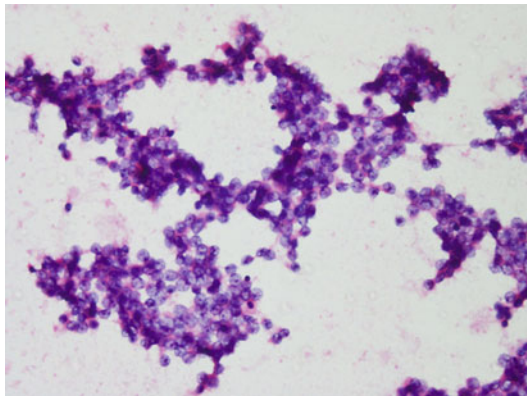

Fig. 3.21 FNAC in a case of adult granulosa cell tumor (H&E x40X)

nuclei which lack nuclear grooves. Foci containing theca cells are seen.

Both AGCT and JGCT show immuno-positivity for CD99, inhibin, and calretinin.

Thecoma

Thecoma occurs over a wide age range, but most patients are peri- or postmenopausal. These can be small tumors to large solid masses with a well-defined capsule. The cut section is typically yellowish with foci of hemorrhage and necrosis. On microscopy, these are usually cellular tumors composed of fascicles of plump spindle cells. The tumor cells have fusiform nuclei, bland chromatin, and abundant intracytoplasmic fat, the last being positive with oil-red O. Hyaline plaque are often conspicuous. On reticulin staining, the reticulum fibers typically surround individual tumor cells, which is a helpful feature to differentiate it from AGCT.

Fibroma

Fibroma is the most common sex cord-stromal tumor. It is a benign tumor which accounts for 1–5% of all ovarian tumors. The clinical symptoms can be nonspecific and rarely are associated with Meigs' syndrome. Grossly, these vary from small in size to very large in size. On cut section, these are solid, hard tumors with a whorled appearance. Microscopically, they are composed of closely packed spindle cells associated with varying amounts of hyalinization and collagenization.

Sertoli-Leydig Cell Tumor

The Sertoli-Leydig cell tumor is a rare tumor. Most occur in young women, and about 50% of these tumors are hormonally active. The most common presentation includes signs of virilization, which develop in about one-third of the cases. Grossly, these are usually solid neoplasms with yellow to tan on the cut surface. Cystic components can be variable. On microscopy, these have been divided into six subtypes: well-differentiated, intermediate differentiation, poorly differentiated, tumors with heterologous elements, retiform, and mixed. Well-differentiated tumors usually have well-defined tubules and trabeculae composed of columnar sertoli cells. Leydig cells are large polygonal cells with eosinophilic cytoplasm. The prognosis of this tumor is usually good and depends upon the stage and the degree of differentiation of the tumor.

Rarely, lymphoma or soft tissue tumors may be seen involving the ovaries.

Metastatic Tumors

The most common primary sites for metastatic tumors are the breast, uterus, stomach, colon, and gallbladder. Krukenberg tumor is an ovarian neoplasm, which is characterized by bilateral nodular ovarian enlargement and shows sheets of signet-ring cells on microscopy.

Carcinoma of Fallopian Tube

Fallopian tubal malignancies account for about 1% of all genital tract malignancies.

It is recently established that tubal fimbria is the main site for serous carcinogenesis. Tubal fimbrial end shows foci of strong p53 immunostaining, termed as "p53 signature" in benign appearing mucosa, and serous tubal intra-epithelial carcinoma (STIC) lesions, which are supposed to be the site of origin for high-grade pelvic serous carcinoma (high grade ovarian serous carcinoma). The majority of patients are postmenopausal and present with vaginal bleeding, vaginal discharge, pelvic pain, or a pelvic mass. Grossly, the tube is

swollen due to a papillary or solid growth filling the tubal lumen. The fimbriated end is closed in about 50% cases. On microscopy, the most common type is papillary serous type, others being endometrioid, clear cell, squamous, small cell, lymphoepithelial-like, or transitional type. The prognosis mainly depends upon the stage of the tumor.

References

1. Ferlay J, Shin H-R, Bray F, Forman D, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 2010;127:2893–917.
2. Arulponni TR, Janaki MG, Nirmala S, Ramesh BS, Rishi KS, Kirthi K. Carcinoma cervix treated with radiotherapy—our experience with emphasis on our concern. J Obstet Gynaecol India. 2010;60:61–5.
3. Ostor AG. Natural history of cervical intraepithelial neoplasia: a critical review. Int J Gynecol Pathol. 1993;12:186–92.
4. Holowaty P, Miller AB, Rohan T, et al. Natural history of dysplasia of the uterine cervix. J Natl Cancer Inst. 1999;91:252.
5. Colgan TJ, Lickrish GM. The topography and invasive potential of cervical adenocarcinoma in situ, with and without associated squamous dysplasia. Gynecol Oncol. 1990;36:246–9.
6. Denehy TR, Gregori CA, Breen JL. Endocervical curettage, cane margins, and residual adenocarcinoma in situ of the cervix. Obstet Gynecol. 1997;90:1–6.
7. Ursin G, Pike MC, Preston-Martin S, et al. Sexual, reproductive, and other risk factors for adenocarcinoma of the cervix, results from a population based case– control study. Cancer Causes Control. 1996;7:391–401.
8. Gupta N, Srinivasan R, Rajwanshi A. Functional biomarkers in cervical precancer: an overview. Diagn Cytopathol. 2010;38:618–23.
9. Gupta N, Desai M. New technologies in cervical cancer screening. BIDA AGM. 2011;18:5–6.
10. Ridder R, Luyten A, Scherbring S, Reinecke-Luettge A, Schmidt D, Petry KU. Triaging PAP negative, HPV positive cervical cancer screening test results by p16/Ki67 dual stained cytology. Acta Cytol. 2010; 54(3):404–5.
11. National Institute for Clinical Excellence (NICE). NHS Technology Appraisal Guidance 69. Guidance on the use of liquid-based cytology (LBC) for cervical screening. London: National Institute for Clinical Excellence; 2003.
12. Utagawa ML, Pereira SM, Makabe S, et al. Pap test in a high risk population comparison of conventional and liquid-based cytology. Diagn Cytopathol. 2004; 31:169–72.
13. Strander B, Andersson-Ellstrom A, Milsom I, et al. Liquid-based cytology versus conventional papanicolaou smear in an organized screening program. Cancer Cytopathol. 2007;111:285–91.
14. Schledermann D, Ejersbo D, Hoelund B. Improvement of diagnostic accuracy and screening conditions with liquid-based cytology. Diagn Cytopathol. 2006;34:780–5.
15. Confortini M, Bergeron C, Desai M, et al. Accuracy of liquid-based cytology. Cancer. 2010;118:203–8.
16. Siebers AG, Klinkhamer PJJM, Grefte JMM, et al. Comparison of liquid-based cytology with conventional cytology for detection of cervical cancer precursors. JAMA. 2009;302:1757–64.
17. Farthing A, Masterson P, Mason WP, Vousden KH. Human papillomavirus detection by hybrid capture & it's possible clinical use. J Clin Pathol. 1994;47:649–52.
18. Ergünay K. Detection and typing of human Papilloma virus by polymerase chain reaction and hybridization assay in cervical samples with cytological abnormalities. Mikrobiyol Bull. 2008;42:273–82.
19. Hubbard RA. Human papillomavirus testing methods. Arch Pathol Lab Med. 2003;127:940–5.
20. Gupta N, Dudding N, Smith JHF. Cytomorphological features of extra-genital metastases in SurePath™ cervical liquid-based cytology: a series of eight cases. Cytopathology 2012; doi: 10.1111/j.1365-2303.2011.00945.x.
21. Liu FS. Molecular carcinogenesis of endometrial cancer. Taiwan J Obstet Gynecol. 2007;46:26–32.
22. Sherman ME, Bur ME, Kurman RJ. p53 in endometrial cancer and its putative precursors: Evidence for diverse pathways of tumorigenesis. Hum Pathol. 1995;26:1268–74.
23. Mutter GL. PTEN, a protean tumor suppressor. Am J Pathol. 2001;158:1895–8.
24. Matias-Guiu X, Catasus L, Bussaglia E, et al. Molecular pathology of endometrial hyperplasia and carcinoma. Hum Pathol. 2001;32:569–77.
25. Cherkis RC, Patten Jr SF, Andrews TJ, Dickinson JC, Patten FW. Significance of normal endometrial cells detected by cervical cytology. Obstet Gynecol. 1988;71:242–4.
26. DuBeshter B, Warshol DP, Angel C, Dvoretsky PM, Lin JY, Raubertas RF. Endometrial carcinoma: the relevance of cervical cytology. Obstet Gynecol. 1991;77:458–62.
27. Ronneett BM, Zaino RJ, Ellenson LH, Kurman RJ. Endometrial carcinoma. In: Kurman RJ, editor. Blaustein's pathology of the female genital tract. 5th ed. New Delhi: Springer; 2004. p. 501–59.
28. Gupta N, Awasthi A, Rajwanshi A, Malhotra S. Fine needle aspiration cytology of low grade endometrial stromal sarcoma—a case report. Acta Cytol. 2007;51(3):461–3.
29. Scully RE, Bonfiglio TA, Kurman RJ, Silverberg SG, Wilkinson EJ. World Health Organization international histological classification of tumors: histological typing of female genital tract tumors. 2nd ed. Berlin: Springer; 1994.
30. Auersperg N. Origin of ovarian carcinomas: a unifying hypothesis. Int J Gynaecol Pathol. 2010;30: 12–21.

31. Murthy NS, Shalini S, Suman G. Changing trends in incidence of ovarian cancer—the Indian scenario. Asian Pac J Cancer Prev. 2009;10:1025–30.
32. Layfield LJ, Berek JS. Fine-needle aspiration cytology in the management of gynecologic oncology patients. Cancer Treat Res. 1994;70:1–13.
33. Kurman RJ, Shih leM. The origin and pathogenesis of epithelial ovarian cancer—a proposed unifying theory. Am J Surg Pathol. 2010;34:433–43.
34. Partridge EE, Phillips JL, Menck HR. The National Cancer Data Base report on ovarian cancer treatment in United States hospitals. Cancer. 1996;78:2236–46.
35. Komiyama S, Aoki D, Tominaga E, Susumu N, Udagawa Y, Nozawa S. Prognosis of Japanese patients with ovarian clear cell carcinoma associated with pelvic endometriosis: clinicopathologic evaluation. Gynecol Oncol. 1999;72:342–6.
36. Yoshikawa H, Jimbo H, Okada S, Matsumoto K, Onda T, Yasugi T, Taketani Y. Prevalence of endometriosis in ovarian cancer. Gynecol Obstet Invest. 2000;50:11–7.
37. Ramalingam P, Malpica A, Silva EG, Gershenson DM, Liu JL, Deavers MT. The use of cytokeratin 7 and EMA in differentiating ovarian yolk sac tumors from endometrioid and clear cell carcinomas. Am J Surg Pathol. 2004;28:1499–505.
38. Dharan M. Intra-operative cytology of small cell carcinoma of the ovary with hypercalcemia: a case report. Acta Cytol. 1993;37:61–6.