

Course AP104

Recognizing the High Risk “Benign” Endometrial Biopsy

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**College of American Pathologists
CAP’11 The Pathologists’s Meeting
Grapevine, Texas**

**Sunday, September 11 2011
2:00pm-5:30pm**

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ABSTRACT

Recognizing the High Risk “Benign” Endometrial Biopsy

NEW

Date/Time TBD

MK PC

3.0 CME

Nonmalignant endometrial biopsies and curettages, frequent components of any busy surgical pathology practice, often present difficult diagnostic challenges due to artifact and morphologic variation amongst individual entities. This course will present practical approaches to resolve commonly encountered diagnostic thresholds with immediate clinical management implications. Topics will include: Metaplasias – benign vs high risk?; Hyperplasias – hormonal effect or precancer?; Things you can miss if you do not think of them; Useful special studies; and Hints for managing the indeterminate case. Special attention will be paid to consideration of sampling problems in a fragmented specimen format, the modifying effects of hormonal environment on interpretation, and those diagnostic thresholds that correspond to clinical management decisions.

You will learn to:

- Identify unusual presentations of commonly encountered non-neoplastic specimens, including sampling and interpretive limitations in their diagnosis
- Describe the features of premalignant endometrium and its distinction from benign mimics and carcinoma
- Resolve benign, premalignant, and malignant subsets of endometrial “metaplasias” and “hyperplasia”
- Describe uses of special studies in common diagnostic settings
- Understand the clinical implications of common pathologic diagnoses

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COURSE DESCRIPTION:

Nonmalignant endometrial biopsies and curettages, frequent components of any busy surgical pathology practice, often present difficult diagnostic challenges due to artifact and morphologic variation amongst individual entities. This course will present practical approaches to resolve commonly encountered diagnostic thresholds with immediate clinical management implications. The course will be organized as a series of 20-30-minute topical presentations, each covering a particular diagnostic problem area of high clinical relevance. Each session will begin with a diagnostic problem with implications for patient management, preceeded by identifying features useful for practical resolution of the differential diagnosis, and then illustrate application in difficult real-world examples. Two types of cases will be included: classic diagnostic exemplars showing readily ascertained diagnostic features, and subtle or ambiguous cases previously identified by their ability to incite diagnostic discordance amongst reviewing pathologists. In the ambiguous cases, available clinical outcomes, and additional studies will provide a measure of appropriate diagnosis.

Emphasis will be placed on commonly encountered diagnostic entities. Special attention will be paid to consideration of sampling problems in this fragmented specimen format, the modifying effects of hormonal environment on interpretation, and those diagnostic thresholds that correspond to clinical management decisions.

Topical Coverage: 4-5 topics will be selected each year and rotated annually

1. Metaplasias – benign vs high risk?
2. Hyperplasias – hormonal effect or precancer?
3. Things you can miss if you do not think of them. (perforation, pregnancy, EIC)
4. Useful special studies: stains and beyond.
5. Limits of Resolution: Hints for managing the indeterminate case. (including sampling errors, artifacts, and confounding factors).
6. Who is at risk for cancer?

Course Outcomes:

- Identify unusual presentations of commonly encountered non-neoplastic specimens, including sampling and interpretive limitations in their diagnosis
- Summarize the differences between endometrial hormonal effects, polyps, and premalignant lesions.
- Describe the features of premalignant endometrium and its distinction from benign mimics and carcinoma.
- Resolve benign, premalignant, and malignant subsets of endometrial “metaplasias” and “hyperplasia”
- Describe uses of special studies in common diagnostic settings.

Understand the clinical implications of common pathologic diagnoses, and use that information to guide the diagnostic process.

COURSE SCHEDULE

Sunday, September 11
2:00pm-5:30pm

- 2:00-2:30 Hyperplasias – hormonal effect or precancer?
Dr. Mutter
- 2:30-3:00 Metaplasias – benign vs high risk?
Dr. Nucci
- 3:00-3:30 Neoplastic Things you can miss if you do not think of them.
(EIC, Cx vs EM, Mets, stromal tumors)
Dr. Nucci
- 3:30-4:00 Coffee Break (Coffee served by CAP)
- 4:00-4:30 Non-Neoplastic Things you can miss if you do not think of them.
(perforation, pregnancy, infection-inflammatory, adequacy, hormonal Rx,
breakdown/artifact(exfoliation), floats
Dr. Mutter
- 4:30-5:30 Virtual Cases. Challenging Cases from Daily Practice.
Drs. Nucci and Mutter

Hyperplasias – hormonal effect or precancer?

George L. Mutter, MD

INTRODUCTION

The diagnostic strategies outlined here are based upon an integrated picture of endometrial carcinogenesis from diffuse hormonal changes (benign hyperplasia sequence) to the earliest recognizable premalignant cells (Endometrial Intraepithelial Neoplasia, EIN) ¹. In the past, both generalized hormonal responses and localized premalignant lesions were lumped under the term “endometrial hyperplasia,” with various modifiers such as “adenomatous”, “mild, moderate, and severe”, and “atypical” that had no uniform meaning. The WHO 1994 classification system subdivided hyperplasias by architectural complexity and cytologic atypia ². Although this practice has been widespread, and has had a benefit of unifying terminology, it fails to optimally stratify patients according to those pathologic mechanisms and cancer risks necessary for appropriate therapeutic triaging. Diagnoses are poorly reproducible ³. Recent molecular studies have provided evidence that the use of the term hyperplasia is conceptually correct for some but not all of these lesions. For these reason, we have chosen to present a practically oriented disease classification in which the hormonal effects of unopposed estrogens (benign hyperplasia) and emergent neoplastic precancerous lesions (endometrial intraepithelial neoplasia (EIN)) are separately diagnosed using non-overlapping terminology and discrete criteria ⁴.

Table I: Endometrial Diagnostic Schema and ICD9 Codes

Nomenclature	Topography	Functional Category	Treatment	ICD9 Code
Benign Endometrial Hyperplasia	Diffuse	Prolonged Estrogen Effect	Hormonal therapy, Symptomatic	621.34
EIN, Endometrial Intraepithelial Neoplasia	Focal progressing to diffuse	Precancerous	Hormonal or surgical	621.35
Endometrial adenocarcinoma, endometrioid type, well differentiated	Focal progressing to diffuse	Malignant	Surgical stage-based	182.0

Part I: The Disordered Proliferative And Benign Endometrial Hyperplasia Sequence

Benign endometrial hyperplasias⁵ do not have a singular histopathologic appearance, but rather demonstrate sequential changes occurring in a combination and severity that reflects the quantity and duration of unopposed estrogen exposure⁶. Characteristic histologic features include irregular remodeling of glands, variably accompanied by vascular thrombi, stromal breakdown and randomly scattered cytologic changes. Some estrogen induced changes persist, with modification, even after the estrogen level declines or is quenched by addition of progestins. This aggregate group of benign endometrial hyperplasias can thus be envisioned as a temporal sequence of estrogen-induced changes in which the appearance at any single time point is codetermined by the trajectory of prior morphologic changes and the current hormonal environment. Prolonged estrogen exposure unmitigated by opposing progestins confers a modest 2-10 fold increased endometrial cancer risk⁷⁻⁹. Those benign endometrial hyperplasias that develop histologically discontinuous EIN lesions are associated with dramatically increased cancer risk. The challenge to the pathologist is to divide the diverse histologic presentations of benign endometrial hyperplasia into functionally defined subgroups, while maintaining a sharp diagnostic boundary with premalignant EIN lesions.

Pathophysiology

Benign endometrial hyperplasia is encountered most frequently around the time of the menopause, when the normal cycle of sequentially regulated estrogen and progesterone is perturbed in tempo and amount. It can also occur, however, in young women and teenagers, in whom anovulatory cycles are also the norm. The primary pathology in all these cases is a systemic excess of estrogens, albeit one in which the endometrium is secondarily altered and a frequent source of symptomatic bleeding. The pathognomonic feature of persistent estrogen stimulation is architectural changes of individual glands distributed randomly throughout the entire hormonally responsive region of the endometrium (superficial functionalis). Prolonged proliferation as a result of unopposed estrogens first gives rise to disordered proliferative endometrium, and over time an increasingly irregular distribution of individually variable endometrial glands which are known as benign hyperplasia. Disordered proliferative endometrium and the earliest phases of benign hyperplasia of the endometrium thus share a common pathogenesis, and present a continuous spectrum of overlapping histopathologic features (Table II) rather than sharply different appearances. Precise discrimination is somewhat arbitrary.

In a woman of childbearing age, there is characteristically prolonged or excessive bleeding at intervals that are initially longer than normal. Microinfarcts and estrogen withdrawal are responsible for symptomatic bleeding^{10;11}. Both mechanisms may be effective at different times in patients with benign hyperplasia. Patchy stromal breakdown secondary to estrogen-induced microthrombi can produce intermittent spotting. A relative reduction in the prolonged estrogen stimulation causes apoptosis of the endometrial glands and stroma of the hypertrophied functionalis¹¹, and resultant heavy shedding. Occasionally, decline in estrogen levels is sufficiently gradual that generalized apoptosis and shedding fail to take place.

Superimposition of progesterone upon a benign endometrial hyperplasia occurs in women with delayed ovulation, sporadic corpus luteum development in the perimenopausal years, or therapeutic administration of progestins following an extended follicular phase. Down-regulation of estrogen receptors by progestins leads to a dominant progestational effect, regardless of the presence or absence of continued estrogen production. In this environment menstrual shedding is delayed, as progestins have the capacity to directly support the endometrium. Progesterone related stromal and secretory

glandular changes develop within the setting of irregular glands previously developed under the influence of estrogens. Thus, the histologic appearance at diagnosis may be heavily modified by intermittent or accompanying progestins although the causal event in benign hyperplasia is unopposed estrogen.

Table II: Histological Features of Benign endometrial hyperplasia (not all are present in every case)

Feature	Comment	Disordered Proliferative	Benign Hyperplasia		Benign Hyperplasia with superimposed progestin effect	Shedding following Benign Hyperplasia
			active phase	exhausted phase		
mitotic activity	similar to normal proliferation	+	+			
scattered cysts	within functionalis, random placement	+	+	+	+	
tubal metaplasia	randomly involves scattered tubular or cystic glands. +/- cilia	+	+	+	+	
variable gland density	“regularly irregular” secondary to gland proliferation and remodeling		+	+	+	
bulky specimen	reflects prolonged proliferative activity		+	+	+	
fibrin thrombi	often separate or displaced		+	+	+	+
microinfarcts with epithelial change	randomly placed, multifocal, with intervening intact		+	+	+	
low or absent mitoses	reflects decline in estrogen			+	+	+
secretory change	variable extent depending on exposure				+	+
stromal pre-decidualization	may be patchy or lacking, depending on progestin exposure				+	+
global breakdown	architectural clues obscured, cytology degenerative					+

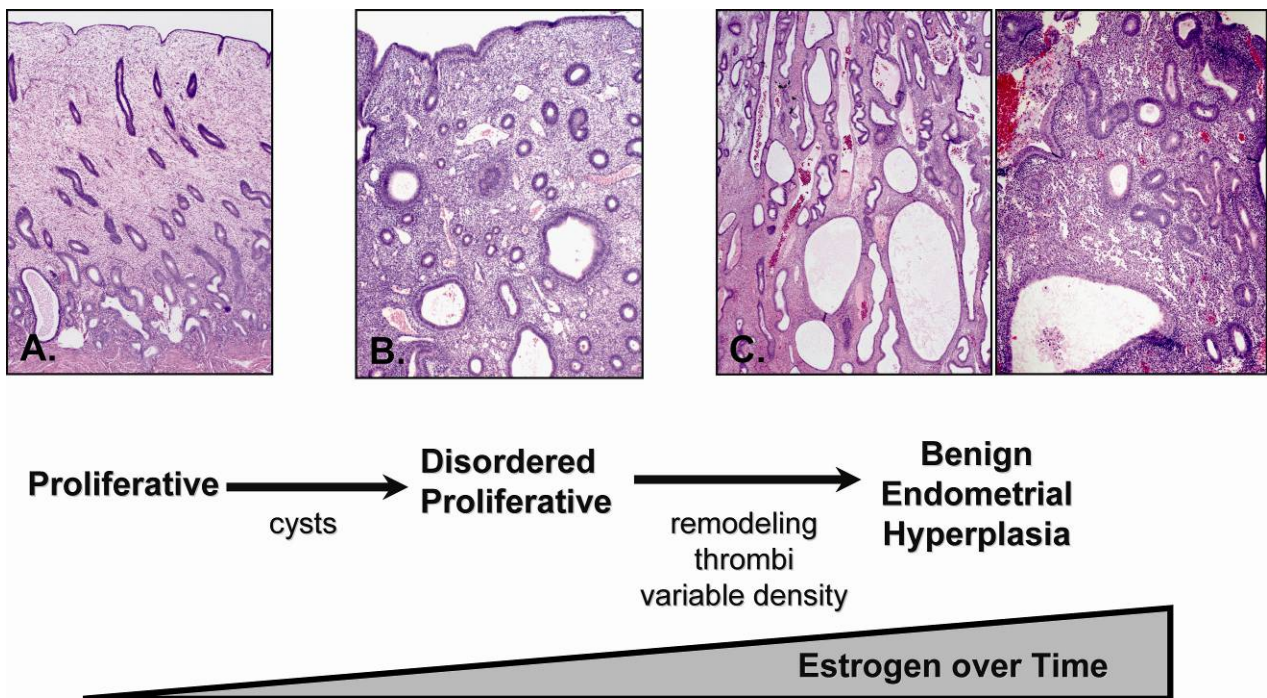


Figure 1: Progressive Effects of Unopposed Estrogens.

Early effects of unopposed estrogen are scattered cysts in an otherwise normal appearing proliferative endometrium, known as disordered proliferative endometrium. Continued exposure causes a progressive spectrum of histopathologic change (left to right) including increasing irregularity of gland density and shape, scattered alterations of cytologic appearance known as benign hyperplasia. Established benign hyperplasias demonstrate a high degree of remodeling between glands and stroma of the expanded, hyperplastic, endometrial compartment, in which the ratio of glands to stroma exceeds 1.0 in most or all of the endometrial compartment. Fibrin thrombi, stromal breakdown and associated reactive epithelial changes commonly develop, and must be carefully distinguished from neoplastic processes.

Diagnostic Features

Abundant curettings with characteristically diffuse and widespread morphologic features typify endometria altered by unopposed estrogens. The histologic changes of disordered proliferative and benign endometrial hyperplasia are conceptually and morphologically well represented as a unified disease spectrum, separate and discontinuous from EIN. The histologic hallmark of the benign hyperplasias is a generalized but non-uniform proliferation of architecturally variably shaped glands that equal or exceed the quantity of the stroma.

Disordered proliferative endometrium.

Disordered proliferative endometrium is an exaggeration of the normal proliferative phase without significant increase in the overall ratio of glands to stroma. The changes involve the entire endometrial compartment, and are evident at low magnification as sacculated dilations (microcysts) randomly scattered amongst tubular glands lined by mitotically active epithelial cells. The stroma is usually dense, cellular and abundant, and mitoses may also be encountered. Some background tubular glands are slightly irregular and minimal budding and branching is commonly seen. Ciliated cell change (tubal metaplasia) of endometrial glandular cells is common, reflecting estrogen's pivotal role in the process. The estrogen primed cell often has substantial cytoplasm. Characteristically, glands

affected by tubal differentiation are randomly interspersed amongst proliferative glands, and they also may demonstrate tubular, branching, or cystic architecture.

Benign endometrial hyperplasia.

Benign endometrial hyperplasia develops from disordered proliferative endometrium under the continued influence of unopposed estrogens. The entire endometrial compartment contains variable gland densities caused by remodeling of stroma and glands to the extent that in some areas the gland to stroma ratio exceeds 1:1. It is the increased gland density that distinguishes benign hyperplasia from disordered proliferative endometrium. Individual glands may be tubular, cystic, or branching, and these forms are commingled throughout. On a large scale the endometrium appears uniformly affected, however, at medium magnification local admixtures of individually variable glands present quite differing appearances among separate microscopic fields. This combination of low magnification uniformity, made up of variable medium magnification fields, can be described as “regularly irregular”.

A critical feature of benign hyperplasia is that the cytology does not change between architecturally crowded and uncrowded areas. This reflects the systemic hormonal etiology of the process that similarly exposes the entire endometrium, and allows its distinction from EIN. Cytologic characteristics may change over time with the evolving hormonal state of the patient, and superimposition of local factors such as breakdown and repair. During the established phase of active estrogen exposure glands are proliferative and interposed tubal metaplasia is common.

Unopposed estrogen states are the most common setting in which fibrin thrombi are seen in the intact endometrial functionalis¹⁰. Fibrin thrombi are rarely seen in normal late secretory endometrium, and there is little evidence that vascular thrombosis is a primary mechanism of normal menstrual shedding. Sometime after initiation of cystic gland dilatation the endothelial lining of ectatic superficial endometrial vessels becomes damaged and occlusive luminal fibrin thrombi form. Thrombi are often intimately associated with discrete areas of surrounding stromal breakdown, which has been interpreted either as a cause or effect of the vascular lesion. Whatever the sequence and mechanism of events, the two are linked in disordered proliferative endometrium and benign hyperplasias, and are responsible for patchy non-synchronous endometrial breakdown and resultant symptoms of spotting and intermenstrual bleeding. Collapse of intervening broken-down stroma may lead to close apposition of endometrial glands, degenerative epithelial changes, and dislodgement of vascular thrombi from their tissue context.

Estrogen production from persistent follicles or by peripheral conversion following the menopause is inconstant. When the estrogen level declines slowly, massive breakdown does not occur and the glands lose mitotic activity. These endometria retain the architectural features of a bulky endometrium with altered gland architecture, but the glands demonstrate a mitotically inactive and non-stratified appearance and may be karyorrhectic. With waning estrogen levels, endometrial bulk declines towards an atrophic pattern, sometimes with cysts.

Differential diagnosis of Benign Endometrial Hyperplasia

A commonly encountered pattern that may be mistaken for benign endometrial hyperplasia is composed of prominent cystically dilated glands with flimsy walls composed of scant fibrous stroma. The terms ‘cystic atrophy’ or ‘cystic atrophic endometrium’ describe these lesions, which show cuboidal or flattened and inactive cells lining the distended glands. Furthermore, the glands in cystic atrophy lack budding and infoldings.

Endometrial polyps may have many of the features of endometrial hyperplasia, but they are localized lesions with a distinctive stroma. Polyps arise as monoclonal overgrowths of genetically altered endometrial stromal cells with secondary induction of polyclonal benign glands through as yet

undefined stromal-epithelial interactive mechanisms^{12;13}. Thick walled blood vessels and fibrous stroma commonly seen in polyps are lacking in benign endometrial hyperplasia. Because polyps are focal lesions, specimens obtained by undirected biopsy or curettage typically contain commingled normal endometrium with a completely different histologic pattern. This is not the case with benign endometrial hyperplasia where the entire functionalis is affected. Despite these differences, there are individual cases in which the distinction between an endometrial polyp and lesions in the benign hyperplasia sequence can be difficult, and endometrial polyp remains one of the most common causes of an incorrect diagnosis of hyperplasia.

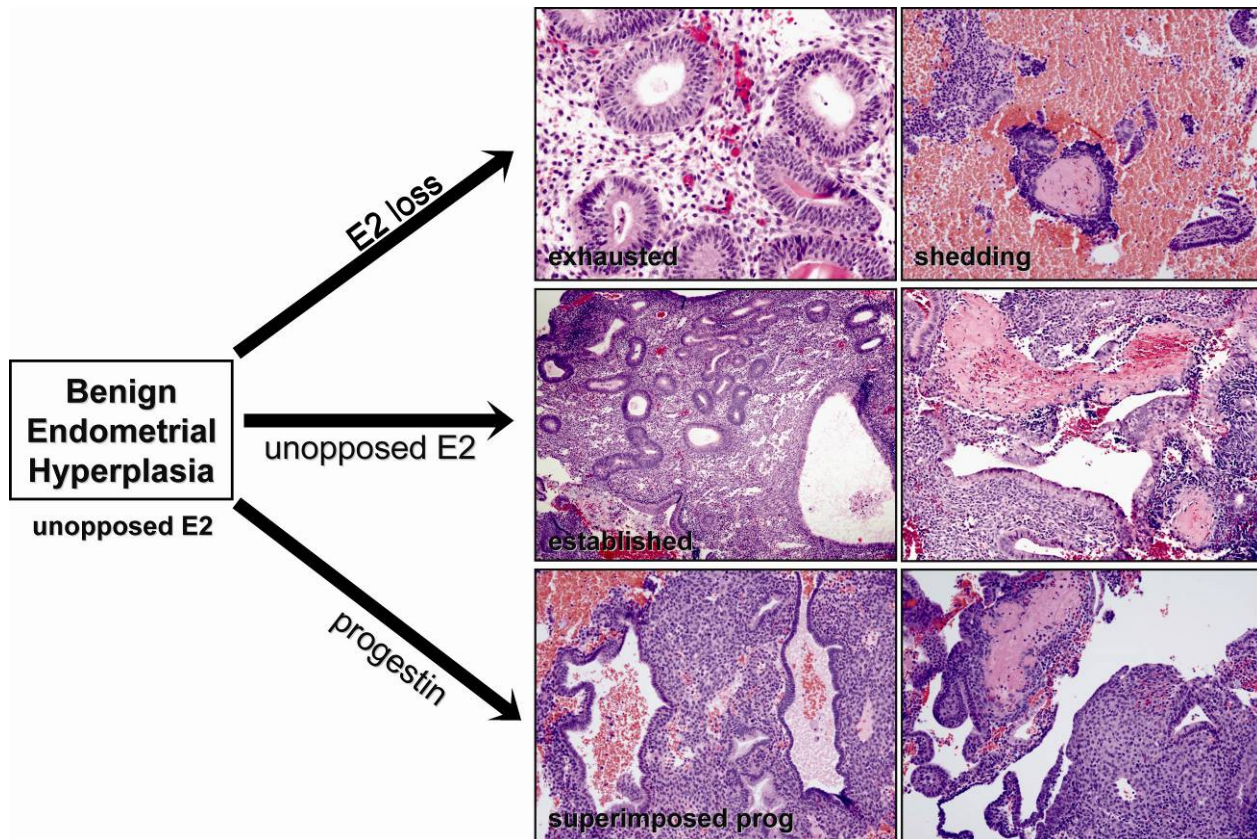


Figure 2: Sequential Modulation of Benign Endometrial Hyperplasia.

Accurate recognition of the class of changes referable to unopposed estrogen, here encompassed within the benign endometrial hyperplasia categories, is facilitated by recognition of their dynamic character and secondary modification within a sequential framework. Cessation or progesterone inhibition of prolonged estrogenic stimulation may occur at any time, at which point benign hyperplasias lose their mitotic activity and the endometrium is no longer proliferative. Architectural changes of the estrogen-driven interval are retained, so that a diagnosis of benign hyperplasia can provide indirect evidence of the prior hormonal state of unopposed estrogens.

Benign endometrial hyperplasia with superimposed progesterin effect.

Superimposition of endogenous or exogenous progestins upon benign endometrial hyperplasia shuts down mitotic activity, and may initiate secretory change with or without subsequent stromal pre-decidualization. The most common endogenous progesterone source is delayed ovulation in a perimenopausal woman, where the corpus luteum is formed on an abnormal schedule, or otherwise is unable to elaborate normal quantities of progesterone. Similar effects can be seen in women having

poor surgical risks, are candidates for hormonal (progestin) therapy. Systemic progestins can successfully ablate up to 90% of endometrial precancers in young women¹⁴, although it is not possible in advance to predict that fraction which will respond. A decision to treat hormonally must thus be made between the clinician and patient in full light of the risks, and with the precondition that regular followup surveillance can be performed.

Figure 3: Clonal Origin of EIN. The first genetic changes (such as PTEN inactivation) which initiate endometrial carcinogenesis are unaccompanied by any phenotypic alterations at the light microscopic level. This “latent”, phase of cytologically and architecturally normal but genetically altered cells may persist for years in a normally menstruating woman. Low cancer risk, combined with lack of a rational therapeutic response, are reasons that systematic screening and treatment of these “latent” phase lesions is unwarranted at present. As additional genetic damage accumulates, higher risk morphologically altered mutant clones declare themselves by demonstrating those architectural and cytologic alterations that distinguish EIN. Malignant transformation of EIN lesions, which occurs at least 46-times more frequently than non-EIN tissues, warrants careful diagnosis and treatment. Endocrine modifiers of endometrial cancer risk act upon the latent and EIN phases of this sequence by tipping the balance of clonal expansion vs. involution.

A combined molecular and histopathologic model for EIN:

Latent, premalignant, and malignant phases of EIN-mediated endometrial carcinogenesis are diagrammed in Figure 3. In almost half of apparently normal women, histologically unremarkable proliferative endometria contain a small fraction of (PTEN tumor suppressor gene) mutant endometrial glands. This phase may be construed as “latent” because not only do the mutated glands look completely normal under the microscope, but they progress to EIN and cancer at very low efficiency. This latent phase may persist for years, with continued presence of scattered and interspersed mutant glands after many menstrual cycles¹⁵. Mutant glands are probably represented in the reserve population of cells that regenerate a new functionalis each month. Endocrine factors act upon these “latent precancers” to modulate involution, or progression to EIN. Transition to EIN requires accumulation of additional genetic damage in at least one “latent precancer” cell, which then clonally expands from its point of origin (indicated by expanding arrows) to form a contiguous grouping of a tightly packed and cytologically altered glands recognizable as EIN. The monoclonal precancer (EIN) develops internal heterogeneity through mutation, and advantageous events selected by local conditions result in hierarchical subclones (left to right) of varying success. EIN lesions have only marginal increases in growth potential, and retain susceptibility to further growth modulation by hormonal factors. Some involute. Others, through additional mutation and selection, reach a stage where hormonal support is no longer required for survival. Malignant transformation to cancer is defined by accumulation of sufficient genetic damage to permit invasion of adjacent stromal tissues. Biomarkers for EIN.

What Is EIN?

Endometrial Intraepithelial Neoplasia, EIN^{19;20}, is the histopathologic presentation of premalignant endometrial disease which confers an elevated risk for endometrial cancer. The singular category of EIN is not stratified or divided into subgroups, and must be distinguished from earlier phases of latent premalignant disease, and endometrial carcinoma. This term was proposed by The Endometrial Collaborative Group¹⁹ to accommodate changing concepts of premalignant endometrial disease and take advantage of revised diagnostic strategies.

EIN needs to be treated, and the type of therapy decided between the patient and treating physician. Things that may influence the choice of surgical vs. hormonal therapy include but are not limited to: diagnostic confidence that a co-existing carcinoma has been excluded, desire for maintained fertility, ability to perform followup surveillance, and patient-specific hormonal and surgical risks.

Clinicopathologic Foundations Of EIN

Rigorous experimental validation of clinically and biologically defined endometrial precancers, and development of correlative diagnostic criteria is a multidisciplinary process. Key predictions expected of precancers which have now been fulfilled for EIN, and practical aspects of their clinical implementation are listed in Table III:

Table III: Precancer postulates fulfilled for EIN

Postulate	Evidence
Precancers differ from normal tissues	Monoclonal ²¹⁻²³ . Divergent genotype ¹⁸ .
Precancers share some, but not all features with carcinoma	Including PTEN ^{16;24;25} , K-ras ²⁶⁻²⁸ , and MLH1 changes ²⁹ . Both are monoclonal ^{21-23;30} . Precancer-cancer lineage hierarchy ¹⁸ .
Precancers can be diagnosed	Computerized morphometry reference standard for EIN ³⁰
Precancers increase risk for carcinoma	High concurrent cancer rate in EIN ^{4;4;31} High future cancer rate in EIN ³²⁻³⁵
Epidemiologic and genetic mechanisms are linked	The PTEN gene, mutated in EIN, is subject to hormonal modulation ^{16;36}
Introducing precancer genotype into an animal produces premalignant lesions and heightened cancer risk	100% of PTEN mutant heterozygote mice get endometrial “hyperplasia” and 21% evolve to carcinoma. ³⁷

Clinical Cancer Outcomes Following EIN Diagnosis

The risk of developing endometrial cancer, as predicted by an EIN diagnosis are the basis for therapy^{31;33;34}. A recent Gynecologic Oncology Group trial of immediate hysterectomy in women diagnosed with EIN on endometrial biopsy or curettage shows 38% (of 148 patients with EIN) had concurrent adenocarcinoma at hysterectomy³⁸. Of the carcinomas seen at hysterectomy, 67% (37/55) had no myoinvasion, 25% (14/55) had myoinvasion within the inner half of the myometrial thickness, and 7% (4/55) had deep myoinvasion to the outer half of the myometrial thickness. These GOG study results are based upon subjective EIN diagnosis.

Other studies show the cancer predictive value of subjective (Figure 5)³² and objective histomorphometric (Figure 6)⁴ EIN diagnosis amongst mixed groups of patients with and without EIN. Subjective EIN diagnosis outperforms cancer prediction when compared to WHO hyperplasia criteria (Fig. 5). Excluding concurrent cancers that are diagnosed within the first year of EIN, patients

with EIN lesions have an overall 45-fold long term increased cancer risk than those without EIN. (Figure 6).

Figure 5: Cancer outcomes (black), by followup interval (vertical axis) of 97 endometrial biopsies diagnosed by WHO hyperplasia (left) or EIN (right) schema³². Endometrial hyperplasias (left panel) were rediagnosed subjectively (without morphometry) as EIN or benign, non-EIN (right panel). All 8 cancer outcomes (black symbols) followed an initial diagnosis of EIN. EIN has a better negative predictive value than atypical hyperplasia, as 2/8 cancer occurrences were seen in the non-atypical hyperplasia groups.

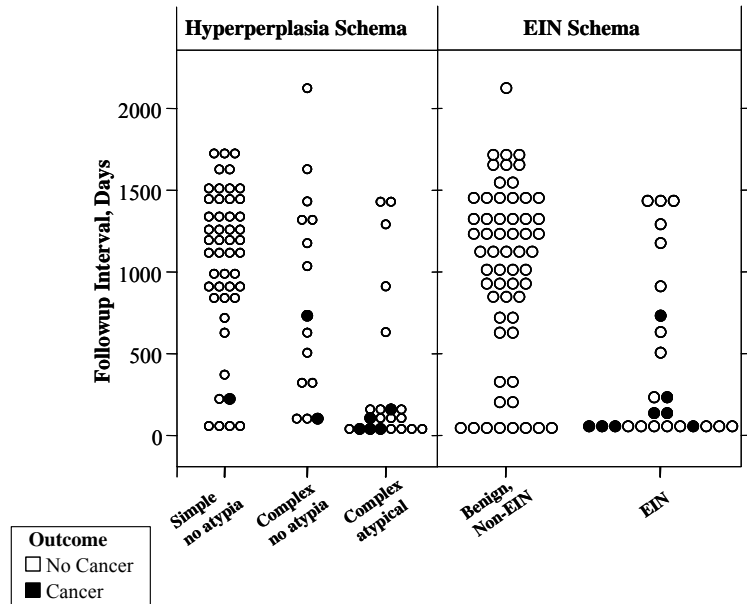
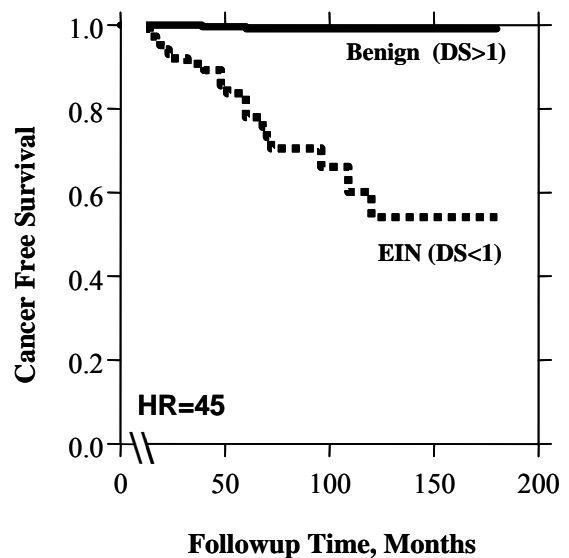


Figure 6: Long term cancer progression in women with EIN⁴. Cancer outcomes that occur more than one year after EIN diagnosis are bona-fide progression events from a premalignant to malignant phase of disease. Progression to cancer more than one year following EIN diagnosis is 45 times more likely compared to women without EIN. Note the tempo of cancer appearance indicates that it can take years for an EIN to evolve into adenocarcinoma.. 477 Women with “endometrial hyperplasia restratified into EIN vs. non-EIN categories. 2/359 non-EIN and 22/118 EIN cases developed adenocarcinoma.



How Is EIN Diagnosed?

⁵(also see www.endometrium.org)

EIN is diagnosed by a pathologist using routine (hematoxylin and eosin stained) sections prepared from a representative endometrial sample^{39,40}. It is extremely important to note that diagnostic accuracy may be severely compromised by exogenous progestin-containing hormonal therapies. For this reason, primary diagnosis or followup surveillance of a suspected EIN lesion should be based whenever possible on a sample obtained while the patient is not on therapeutic hormones. For those patients on progestins, diagnostic tissue can be obtained 2-4 weeks after stopping exogenous hormones, after completion of a withdrawal bleed. Although computerized morphometry has been a useful tool in identifying features characteristic of EIN, such equipment is not required for routine diagnosis. Rather, pathologist interpretation of stated criteria at a standard microscope is adequate.

It should be noted that EIN is a precursor of endometrioid endometrial adenocarcinomas and is unrelated to the "Endometrial Intraepithelial Carcinoma" proposed ⁴¹ to be the earliest stages of papillary serous type endometrial adenocarcinomas.

A framework for EIN Diagnosis is shown in Table I at the beginning of this syllabus. Notable is the clear separation of endometrial changes caused by unopposed estrogens, and carcinoma, from EIN.

1. Topography of EIN

The distribution of a lesion is useful in distinguishing between the diffuse, field-wide effects, of an abnormal hormonal environment (anovulation, or persistent estrogen effect), surface changes secondary to stromal breakdown, and more focal EIN. Clonal origin from a single cell requires EIN lesions to begin as local processes within the endometrial compartment. Early EIN lesions are easily diagnosed by their contrast in architecture and cytology with the background from which they have emerged. Over time, EIN lesions may completely overrun the background endometrium, thereby removing the convenient lesion-to-background contrast in morphology which assist in EIN diagnosis. For this reason, or because of fragmentation, many EIN lesions must be diagnosed without the benefit of comparison with companion benign tissues. Exclusion of artifact and careful evaluation of the architectural and cytologic features of EIN usually permits accurate diagnosis in these instances.

2. EIN Diagnostic Criteria

All of the diagnostic criteria of Table IV, listed as A-E below, must be met in order to make an EIN diagnosis. The entire slide should first be scrutinized under low magnification for localizing lesions, and if found, these areas examined under higher power to assess possible changes in cytology within the architecturally distinct focus. Widespread EIN lesions that have replaced the entire endometrial compartment tend to have a sufficiently atypical cytology that background normal endometrium is no longer required as a reference point for accurate diagnosis.

Size, architecture, and cytology features are easy EIN diagnostic criteria. Much more difficult are exclusion of benign mimics and adenocarcinoma from the differential diagnosis. There are no simple rules for benign mimic exclusion. The broad universe of competing entities can only be recognized on sight by one who has the easy familiarity that comes with experience. Consistent demarcation of the EIN-adenocarcinoma threshold remains important clinically because it provides a basis for the clinician to evaluate the risks of electing hormonal rather than surgical therapy in younger patients who wish to retain fertility.

Special diagnostic challenges, such as recognition of EIN within polyps, interpretation of subdiagnostically small or fragmented lesions, and interpretation of lesions with non-endometrioid differentiation have specific caveats presented below that should be carefully studied.

Table IV: EIN Diagnostic Criteria. Modified after ²⁰.

EIN Criterion	Comments
Architecture	Area of Glands greater than Stroma
Cytology	Cytology differs between architecturally crowded focus and background, or clearly abnormal.
Size >1 mm	Maximum linear dimension exceeds 1mm.
Exclude mimics	Benign conditions with overlapping criteria: Basalis, secretory, polyps, repair, etc..
Exclude Cancer	Carcinoma if mazelike glands, solid areas, polygonal “mosaic-like” glands, myoinvasion, or significant cribriforming

a.Architecture: Gland area exceeds stromal area:

A cardinal architectural feature of endometrial precancers is glandular crowding, with a threshold quantitative cutoff for EIN lesions of less than half of the tissue area occupied by stroma (Volume Percentage Stroma). Areas with large dominant cysts should always be avoided in making this assessment. Although EIN is an epithelial disease, visual assessment of the glands themselves is complicated by frequent artifactual displacement from associated stroma, pale staining of most epithelia, and visual "shimmering" between gland epithelia and lumens. These may all be avoided by focusing on the stromal compartment which has the significant advantages of a more uniform composition throughout the specimen, and superior staining qualities. By focusing on the stroma itself only intact fragments in which stroma has not been avulsed from glands will be evaluated.

Careful review of graphic and histologic examples of varying stromal densities will assist in training your eye to classify patient material as above or below the diagnostic threshold. EIN lesions tend to cluster with a median volume percentage stroma of about 40% and non-EIN (benign) lesions cluster at a median of approximately 75%. These differences are sufficiently great that visual assessment by a trained eye can be informative.

b.Cytology of architecturally crowded area is different than background, or clearly abnormal:

There is no absolute standard for cytologic features of EIN lesions, but the cytology of EIN is usually clearly demarcated as divergent from that of co-existing benign endometrial tissues in the same patient. The manner of cytologic change in EIN varies considerably from patient to patient, and can include but not be limited to, increased variation in nuclear size and contour, clumped or granular chromatin texture, change in nucleoli, change in nuclear/cytoplasmic ratio, and altered cytoplasmic differentiation. Stereotypical static descriptions of cytologic atypia, such as nuclear rounding and appearance of nucleoli are met in many but not all EIN lesions. In this sense, a fixed presentation of cytologic atypia is not a prerequisite for EIN. Attempts to define an absolute standard are confounded by the extreme morphologic plasticity of endometrial glandular cells under changing hormonal, repair, and differentiation conditions.

Cytologic changes in some EIN lesions are manifest as a change in differentiation state to a tubal, mucinous, micropapillary, or eosinophilic phenotype. These must be distinguished from the

scattered random pattern of hormonally, or surface located repair-induced “metaplasias.” Further details of how to interpret non-endometrioid EIN lesions are presented in the “Pitfalls” section below.

In those cases with no normal glands for internal reference, it is necessary to assess the freestanding cytology of relevant fragments in the context of their architectural features. Some EIN lesions occupy the entire tissue sample, and should not be underdiagnosed for lack of a convenient benign gland in the area.

c. Size >1mm in maximum dimension:

Accurate EIN diagnosis requires a contiguous field of glands sufficiently large to enable reliable assessment of architecture. A minimum lesion size of 1 mm maximum dimension was required in the previous clinical outcome studies^{4,31,33,35} for an EIN lesion to achieve elevated cancer risk. That area of an EIN lesion which meets architectural (gland area) and cytologic (changed) criteria for diagnosis must measure a minimum of 1mm in maximum dimension, a scale which usually encompasses more than 5-10 glands. Most biopsy formats produce tissue fragments in excess of 1.5-2mm. The size requirement must be met in a single tissue fragment, not added amongst multiple fragments. There is no formal evidence that once beyond the minimum 1mm, EIN lesions should be stratified by size, but if a lesion is discretely focal, it may be of interest to the clinician to know what fraction of the available curettings contain lesion.

Individual or small clusters of cytologically altered glands have an undefined natural history and are best diagnosed descriptively (See Pitfalls section below).

d. Exclusion of Benign Mimics

Patients with one of the conditions listed below may still have an EIN, but this diagnosis should be made with careful consideration into how the coexisting factor(s) may modify the criteria for EIN diagnosis. If a specimen is refractory to confident diagnosis, a comment as to the nature of the problem may be useful in directing management.

1. **Reactive changes** caused by infection, physical disruption, recent pregnancy, or recent instrumentation. These can cause piling up of the epithelium, and loss of nuclear polarity..
2. **Artifactual gland displacement**. Beware diagnosing an EIN lesion if the cytology is identical between areas with crowded compared to uncrowded glands! Many of these are artifactual disruptions where the stroma is sheared and glands pushed in apposition .
3. **Persistent Estrogen Effect**: Randomly scattered cysts of protracted estrogen exposure and occasional branching glands are commonly encountered in anovulatory or estrogen-exposed endometria. Gland density is uniformly irregular throughout the endometrial compartment, with occasional clusters of glands having a cytology identical to the uncrowded areas. These can be diagnosed as “Benign Endometrial Hyperplasia” if glands are significantly crowded, or in some mild cases as "disordered proliferative" endometrium. With increasing duration, microthrombi form and scattered stromal breakdown may be associated with epithelial piling along the collapsed stromal surfaces.
4. **Mid to late secretory endometrium** displays loss of nuclear polarity, nuclear enlargement, and variation in nuclear size which if measured objectively by computerized morphometry overlaps substantially with EIN lesions. Stromal responsiveness to progesterone is not homogenous at all endometrial depths. Lack of stromal pre-decidualization in the deeper functionalis and superficial basalis makes glands appear crowded, and these same glands may display a worrisome cytology and complicated saw-toothed luminal profiles
5. **Endometrial polyps** contain irregularly spaced glands in which scattered glands may differ from native endometrium due to their tendency to have reduced hormonal responsiveness. Benign polyps may also have low volume percentage stroma caused by cysts (senile polyps) or

random aggregations of glands. Approximately 10% of EIN lesions, however, will present within an endometrial polyp and these must be diagnosed as described below in the “Pitfalls” section.

6. **Endometrial breakdown** is one of the most common settings for overdiagnosis of a benign endometrium as a precancer or cancer. Breakdown may follow an ovulatory or anovulatory cycle and persist into the transitional period between late menses and early proliferative endometrium. Altered cytology is due to piling up of epithelial cells unsupported by stroma, and associated nuclear changes such as loss of polarity which may be accentuated under certain fixation conditions which exaggerate chromatin texture (Bouin's fixative).

e.Exclusion of Carcinoma

Cancer may coexist with EIN in an individual patient, but should be always be separately diagnosed because current management of carcinoma differs from that for EIN. Keep in mind that absence of carcinoma in a tissue biopsy does not exclude the possibility of that the patient has a cancer which was unsampled during the biopsy procedure. An opinion should always be rendered based upon available material, and clearly stated.

EIN is composed of individual glands lined by an epithelium one cell layer thick. The epithelium may be pseudostratified, but should not be cribriform or composed of solid areas of epithelial cells. Presence of any of the following features involving neoplastic glands is inconsistent with EIN, and a diagnosis of carcinoma should be entertained.

1. Meandering or “mazelike” lumens
2. Solid epithelium
3. Cribriform architecture.
4. “Mosaic” gland pattern of distorted polygonal glands with threadlike intervening stroma

Myoinvasion. Unfortunately, myometrium is rarely available for evaluation in a biopsy or curettage specimen.

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Endometrial Metaplasia - Benign vs. High Risk

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Introduction

Endometrial metaplasia is defined as a change in cellular differentiation to an epithelial type that is typically not present in normal cycling endometrium. A variety of alterations in endometrial epithelial differentiation may occur and these changes can be associated with a spectrum of processes ranging from benign to premalignant to malignant. As they can be associated with such a wide range of lesions with varied clinical significance, it is best to recognize the broader diagnostic context rather than simply making the diagnosis of metaplasia. The most common forms of metaplasia are those in which endometrial glands assume a morphology resembling that seen elsewhere in the Müllerian tract, such as the mucus secreting cells (as normally present in the cervix) or the ciliated cells (as seen in the fallopian tube).

Types of endometrial metaplasia include

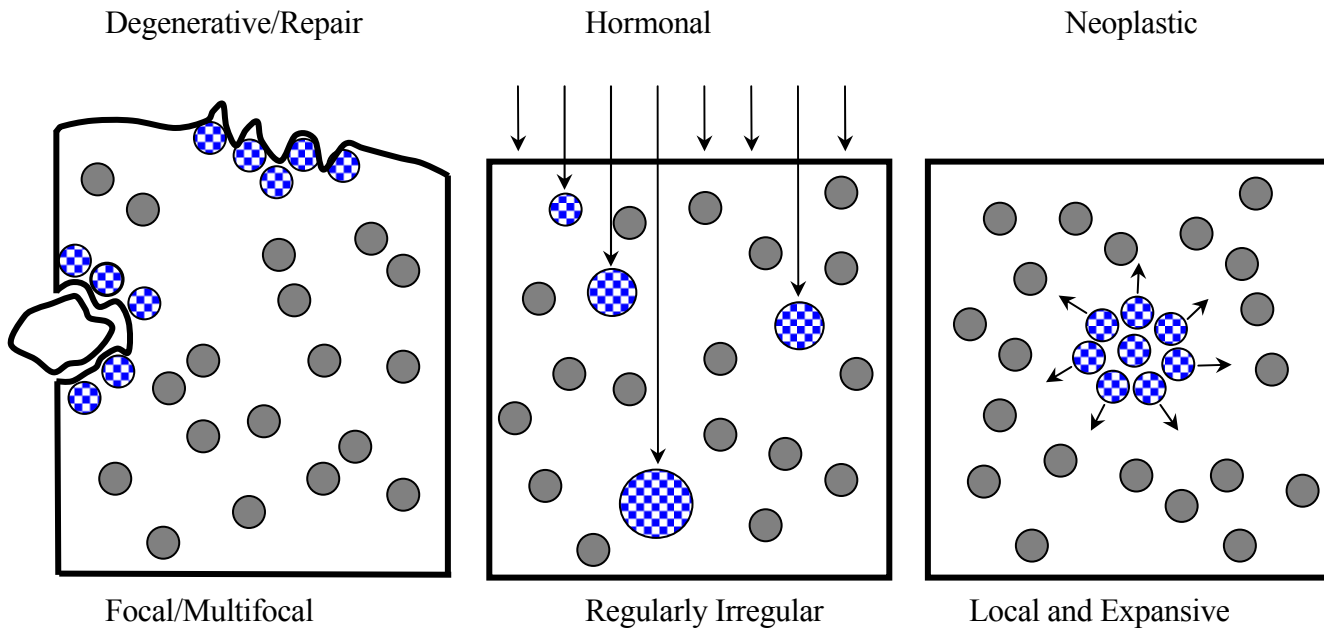
- ▶ Squamous, usually in the form of morules
- ▶ Mucinous
- ▶ Tubal
- ▶ Papillary
- ▶ Eosinophilic

Major Pathogenetic Mechanisms Associated with Metaplasia

It is important to understand that Müllerian derived structures have the capacity to maintain some degree of "plasticity" with regard to differentiation such that any number of pathogenetic mechanisms may incite differentiation that is normally not present for the particular anatomic location. Therefore, the pathologist should always attempt to determine the underlying mechanism, and use diagnostic terminology in their report that clearly communicates the clinical significance of the changes that are present. The three major pathogenetic mechanisms associated with endometrial epithelial metaplasia include:

- ▶ Degenerative/reparative
- ▶ Hormonal
- ▶ Neoplastic.

In general, the topography of the lesion is helpful in recognizing the underlying mechanism (**Figure 1**). Degenerative changes are typically focal or multifocal and can be localized to areas of inflammation, stromal breakdown or recent surface repair. Hormonally induced changes are more commonly diffusely and randomly scattered throughout the endometrial compartment (typically the functionalis or upper 2/3 of the endometrial lining). In contrast, neoplastic (pre-malignant and malignant) processes are monoclonal and start as localizing lesions which expand and sometimes overrun the normal background endometrium.

Figure 1: Major Mechanisms Associated with Endometrial Epithelial MetaplasiaIdentifying High Risk Endometrial "Metaplasia"

Identifying those metaplasias associated with/representing a premalignant and malignant process is the crux of identifying those that are high risk for the patient.

1) Differentiation (Metaplasia) to a Squamous Epithelium

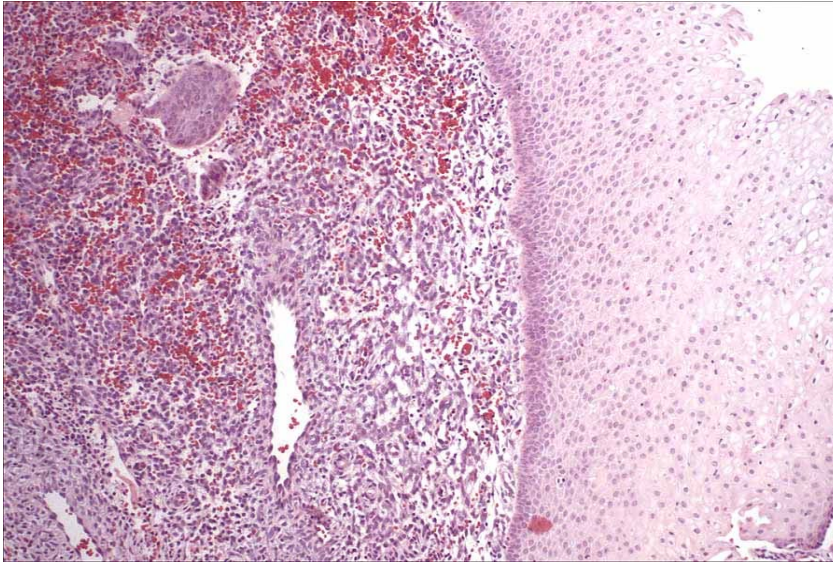
Squamous differentiation in the endometrium can take the form of variably keratinized, stratified squamous epithelium as normally seen lining the exocervix, or as expansile round nests of cells with bland ovoid nuclei and eosinophilic cytoplasm, which have been termed morules. Squamous differentiation occurs in approximately 25% of endometrial adenocarcinomas and many premalignant EIN lesions, but can be seen in benign reactive changes as well. Of particular importance, squamous metaplasia of the morular type is most commonly encountered in a neoplastic setting.

Degenerative/Repair Category - No Risk

- Squamoid change associated with stromal breakdown and surface reparative change
e.g. chronic endometritis, infarcted endometrial polyp, IUD

These changes are typically focal or multifocal and the underlying issue should be the main component of the diagnosis (**Figure 2**).

Figure 2. Surface squamous change associated with chronic endometritis. Patient had an IUD in place.

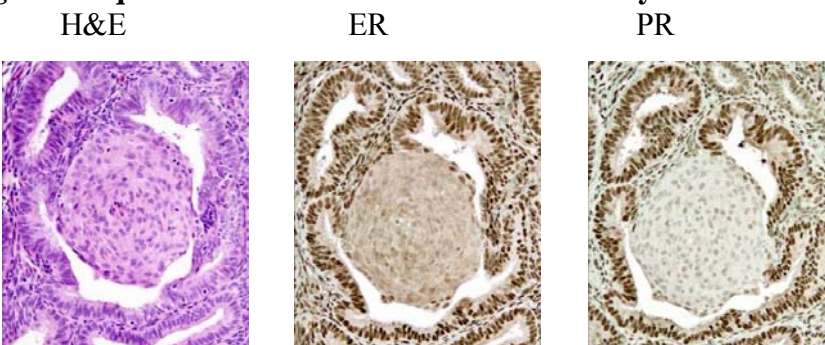


Neoplastic Category - High Risk

- ▶ EIN with squamous morules
- ▶ Atypical polypoid adenomyoma (morules)
- ▶ Adenocarcinoma with squamous differentiation (morules or stratified squamous).

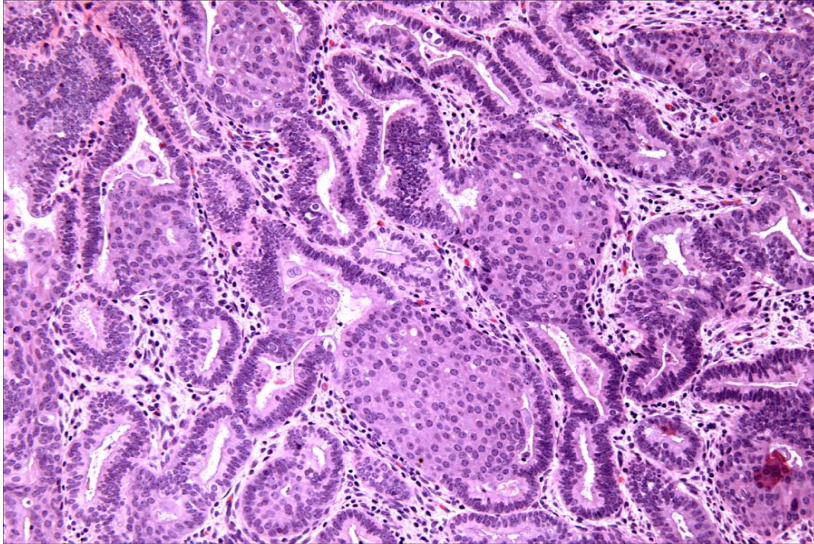
In examining endometrial samples with squamous morules it is important to remember that even though morules may be an intimate component of a premalignant (EIN) lesion, it is the glandular element that has the biologic potential to progress to carcinoma. For this reason, the diagnosis of a premalignant lesion is based on the glandular component only. Even though squamous morules within EINs arise from transdifferentiation of the glandular component (which is supported by the fact that both are clonal and share similar genetic changes), the morules are hormonally inert, which is due to the loss of estrogen and progesterone receptors in this component (**Figure 3**). In addition, squamous morules have minimal mitotic activity and exhibit a low proliferation rate (by Ki67 immunostaining). This finding has clinical implications for the practicing pathologist (and clinician) as EIN lesions with squamous morules treated with progestins, or exposed to progestins during the normal menstrual cycle, may involute, resulting in residual (isolated) squamous morules with little to no accompanying glandular lesion (see *Uncertain Diagnostic Category* below).

Figure 3. Squamous morules in EIN are hormonally inert



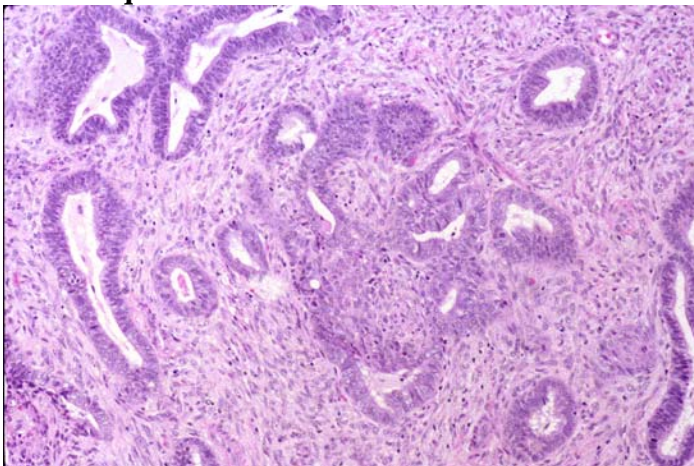
The diagnosis of EIN is based upon established criteria and depends on the degree of architectural crowding within the lesion as well as the difference in appearance (cytologic demarcation) of the epithelium in comparison to the background endometrium (**Figure 4**).

Figure 4. EIN with squamous morules



Prior to making the diagnosis of EIN with squamous morules, or in consideration of any glandular lesion in association with morules, the possibility of an *atypical polypoid adenomyoma* (APA) should be considered. APA is a rare uterine tumor, most commonly involving the lower uterine segment, which consists of architecturally complex endometrial glands embedded in a fibromyomatous stroma (**Figure 5**). APA is distinguished from EIN with squamous morules by the presence of polypoid fragments of tissue and the distinctive stromal component. Distinction from myoinvasive adenocarcinoma is more problematic, but in general lack of separate fragments of non-invasive adenocarcinoma (which would be unusual in an endometrial adenocarcinoma with myoinvasion) and a lesser degree of architectural complexity within the polypoid fragments of the APA (in comparison to the frequent cribriform architecture of an endometrioid adenocarcinoma) aids in this distinction.

Figure 5. Atypical polypoid adenomyoma. Note the prominent fibromyomatous stroma and the central squamous morule.



Uncertain Diagnostic Category - but with risk of subsequent/concurrent neoplasia

- ▶ Isolated squamous morules
- ▶ Morules associated with glandular complexity sub-diagnostic of EIN or carcinoma
- ▶ Ichthyosis uteri

The diagnosis and management of lesions containing squamous morules is dependent on interpretation of the glandular component. While management of lesions classified as EIN is straightforward (by either hormonal or surgical ablation), the diagnosis and management of lesions that are sub-diagnostic due to either lack of a glandular component or a glandular component that does not fulfill architectural or cytologic criteria, are more problematic. These fall into two categories: 1) *isolated squamous morules* and 2) *endometrial glandular proliferations associated with squamous morules* that are difficult to classify. Isolated squamous morules may be encountered as a primary diagnosis or in patients with prior EINs that have been treated with progestins. The risk of subsequent neoplasia is the greatest in those lesions with a glandular component showing some degree of architectural complexity; nevertheless, all patients with squamous morules should have followup sampling as they do have a low, but significant, risk of subsequent neoplasia. In a study by Lin et al, followup endometrial sampling (mean 2 years) in patients with isolated squamous morules and morules associated with gland crowding showed EIN in 6.7 and 28.6% and carcinoma in 3.4 and 14.3% respectively. *Ichthyosis uteri* is a rare lesion in which stratified squamous epithelium replaces the uterine lining epithelium with involvement of the surface and extension into the glands. Numerous bouts of chronic endometritis over a protracted interval are almost always an antecedent condition. Although there is no evidence that ichthyosis uteri is premalignant, we have seen examples of invasive squamous cell carcinoma of the endometrium associated with this lesion.

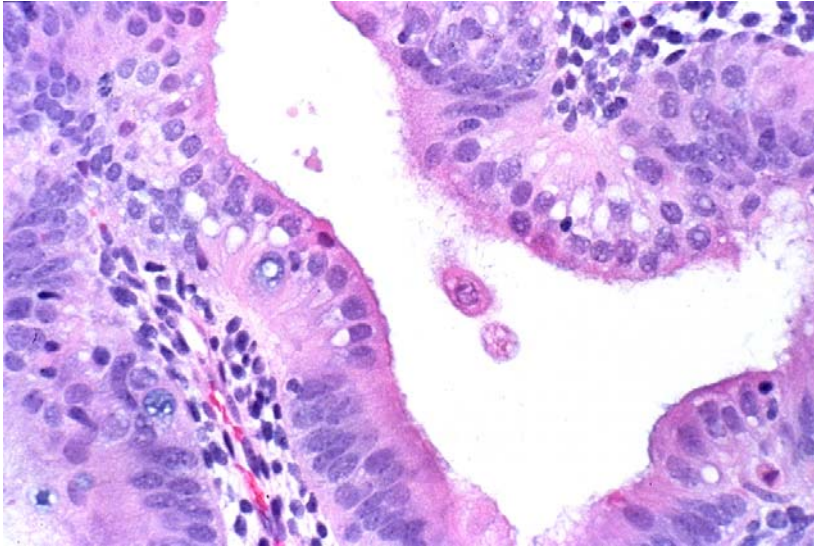
2) Differentiation (Metaplasia) to a Mucinous Epithelium

Mucinous differentiation in the endometrium is relatively frequently encountered and can take the form of either intracytoplasmic mucin droplets or well formed mucinous epithelium, most commonly of the endocervical type. The bland cytologic appearance and close resemblance to endocervical epithelium of some endometrial neoplastic processes makes interpretation of these lesions challenging. Interpretation is particularly problematic when the specimen is scanty and the epithelium is fragmented, which does not allow for evaluation of the underlying architecture.

Degenerative/Repair Category - No Risk

- ▶ Mucinous differentiation associated with syncytial repair and stromal breakdown
- ▶ Endometrial polyp with mucinous differentiation

In general, degenerative processes demonstrate disorganized, variably sized intracytoplasmic mucin droplets and are most commonly seen in association with syncytial repair with stromal breakdown. Only a minor degree of epithelial complexity is present and may take the form of epithelial multilayering and irregular slit-like spaces. Benign mucinous change may also be seen in endometrial polyps or in association with HRT (**Figure 6**) in which simple tubular glands or surface lining epithelium is replaced by a single layer of epithelium with focal intracytoplasmic mucin droplets or rows of tall columnar epithelium with apical mucinous cytoplasm similar to endocervical-type epithelium.

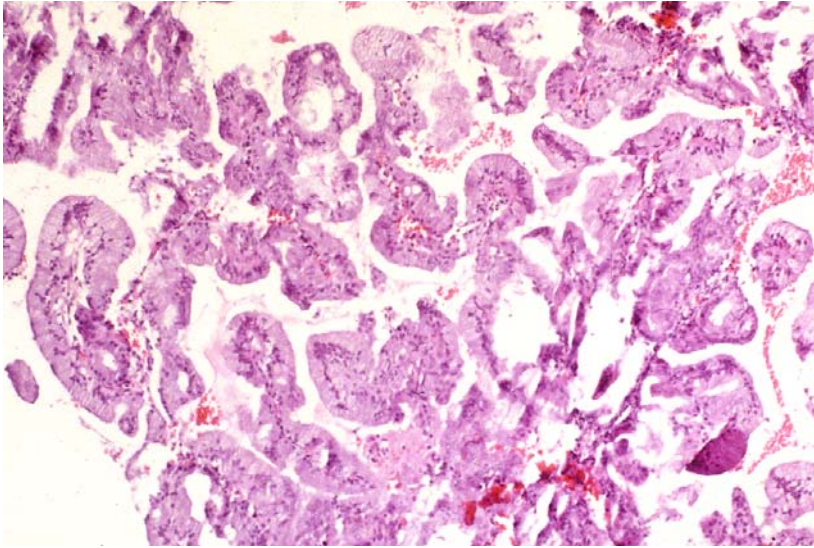
Figure 6. Intracytoplasmic mucin droplets associated with HRT*Neoplastic Category - High Risk*

- ▶ EIN with mucinous differentiation
- ▶ Well differentiated mucinous adenocarcinoma (microglandular-like)

The degree of concern one should have for mucinous differentiation in the endometrium depends primarily on the architecture with worrisome features including gland crowding, exophytic villoglandular growth, and cribriform growth. Papillary mucinous change is a special situation in which the lesion may be benign, premalignant or malignant and its diagnosis is dependent on the location, extent, and type of papillary change. *EIN with mucinous differentiation* is recognized by the crowded focus of glands in which the mucinous differentiation distinguishes it from the background endometrium. The extent of mucinous differentiation in these lesions is variable and can be different between glands and even within an individual gland within the lesion; often EIN lesions with mucinous differentiation blend into areas that have a more endometrioid appearance. Intraglandular non-villous papillae lined by mucinous epithelium confined to the separate, but crowded glands, may also be seen in EIN and are not diagnostic of carcinoma. It is important to realize that focal intraglandular papillary mucinous change can be seen in non-crowded glands within an endometrial polyp and is not diagnostic of a neoplastic process.

Well differentiated endometrial adenocarcinoma with mucinous differentiation is readily distinguished from EIN by the presence of conspicuous villoglandular growth or cribriform architecture. The papillary fronds are supported by delicate branching stromal cores and may appear in biopsy specimens as "free floating" filiform fragments (**Figure 7**).

Figure 7. Well differentiated mucinous adenocarcinoma of the endometrium with a prominent frond-like growth pattern



These fragments may be admixed with a microglandular component or the microglandular component may be the sole indication of malignancy. The *microglandular mucinous adenocarcinomas* are typically composed of a proliferation of bland appearing mucinous epithelium with rigid, "punched out" appearing lumens, which may superficially resemble microglandular change of the cervix. It is usually readily distinguished from the latter by its lack of sub-nuclear vacuoles and reserve cell (squamous) metaplasia.

Uncertain Diagnostic Category - but with risk of subsequent/concurrent neoplasia

Highly fragmented and/or scanty specimens that contain mucinous epithelium with a focally complex papillary or microacinar architecture may or may not represent a well differentiated mucinous adenocarcinoma of the endometrium. This diagnosis is highly dependent on the amount of tissue, its preservation and the degree of fragmentation. In situations in which the findings are not diagnostic of a well differentiated mucinous adenocarcinoma but there is sufficient architectural changes that merit concern, we advocate use of the term "complex mucinous endometrial epithelial proliferation" and advise a followup endometrial sampling in 3-6 months.

Resemblance of some endometrial mucinous lesions to microglandular hyperplasia of the cervix or normal endocervical epithelium can be diagnostically challenging, particularly in scanty specimens. One helpful clue is that normal endocervical epithelium tends to have a uniform appearance to the cytoplasmic mucinous differentiation whereas some mucinous adenocarcinomas of the endometrium have mucinous differentiation that shows a combination of epithelial cells with cytoplasmic mucinous differentiation having both a basophilic and oncocytic appearance. Microglandular change of the cervix also often has a component of squamous differentiation with a layer of basal reserve cells. At the very least, the presence of well differentiated mucinous epithelium within endometrial tissue fragments is a significant abnormality that requires explanation. In some instances, the origin of the mucinous epithelium is not easily discernible and a fractional D&C should be considered.

3) Differentiation (Metaplasia) to a Tubal Epithelium

Tubal differentiation is the most commonly encountered form of endometrial metaplasia accounting for up to 60% of all epithelial metaplasias at this site. While the presence of cilia is the classic manifestation of this type of change, the ciliated cells often also have increased eosinophilic cytoplasm and can be interspersed with secretory type cells as is seen in fallopian tube epithelium. The amount of cilia may vary (with some areas being cilia-poor) and the tubal differentiation may only demonstrate (and be recognized by) the cytoplasmic qualities of the ciliated and secretory cells. Some cilia-poor tubal metaplasias also demonstrate prominent eosinophilic features and may be considered by some to be a mixed type metaplasia (e.g. tubal and eosinophilic). Tubal differentiation in the endometrium may be induced by estrogen exposure or be a manifestation of a neoplastic clone; thus, it can be associated with a spectrum of lesions ranging from benign to premalignant to malignant.

Hormonal Category - No to Minimal Risk

► Benign endometrial hyperplasia (disordered proliferative endometrium)

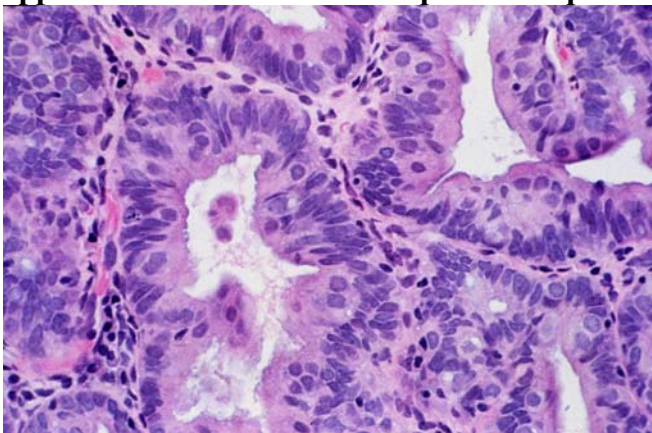
Tubal metaplasia is frequently encountered in non-neoplastic proliferative endometrium, particularly on the surface endometrial lining, suggesting it can be a normal physiologic finding. Prolonged estrogen exposure, as seen with anovulation, is associated with more extensive tubal differentiation that involves the entire endometrial compartment. With longer durations of estrogen exposure, the tubal differentiation is seen scattered amongst the cysts and irregularly spaced glands (disordered proliferative endometrium). Tubal differentiation is also a common finding in endometrial polyps, which also show a distribution of epithelial change that is non-localizing.

Neoplastic Category - High Risk

► EIN with tubal differentiation

Precancerous EIN lesions with localized clonal architecture may also demonstrate tubal differentiation within the affected glands in distinction to the background endometrium (**Figure 8**). Commonly, the EIN lesions have variable ciliated differentiation, small micropapillary projections and exhibit cytoplasmic differentiation reminiscent of the ciliated and secretory cells of the fallopian tube (imparting a round cell - columnar cell alternating pattern).

Figure 8. EIN with tubal differentiation. The cells have an alternating round cell - columnar cell appearance reminiscent of fallopian tube epithelium. The cilia are not conspicuous in this example.



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Neoplastic Things You Can Miss if You Don't Think of Them

Marisa R. Nucci, M.D.

Benign Endometrial Polyp vs Müllerian Adenosarcoma, Low Grade (MA)

One of the most challenging aspects of gynecologic pathology is the recognition of Müllerian adenosarcoma and its distinction from a benign polyp. Most MAs occur in postmenopausal patients, usually in the sixth decade; however, they can occur over a wide age range and the age of the patient is usually not helpful in this distinction. The most common symptoms and clinical findings at presentation for patients with MA include abnormal vaginal bleeding, an enlarged uterus, a pelvic mass or tissue protruding through the cervical os. While the latter two may raise suspicion for malignancy, many patients present with abnormal bleeding, a relatively non-specific symptom that occurs in association with both benign and malignant processes, and certainly one of the most common presenting symptoms for benign polyps. Although many uterine MAs present as bulky, soft, polypoid masses that fill the endometrial cavity, some present as single polypoid lesions involving the uterine fundus and less commonly the lower uterine segment or cervix.

Classic histologic features of MA include:

- Tubular, dilated or cleft-like glands
- Periglandular hypercellular stroma
- Intraglandular polypoid projections
- Benign epithelium with altered differentiation (tubal, mucinous, squamous)
- Stromal mitotic activity > 2 per 10 HPF
- Stromal cellular atypia

The diagnosis of low grade MA is not challenging when all of these features are present; however, not all MAs exhibit all of these classic histologic findings and some benign polyps may have one (or more) of these features.

Benign lesions that mimic MA, low grade

- Endometrial polyp with cellular stroma
- Endometrial polyp with bizarre stromal cells
- Endometrial polyp with adenomyomatous stroma
- Mixed endometrial/endocervical polyp

Endometrial polyps can exhibit a prominent cellular and, depending on the phase of the menstrual cycle, mitotically active stroma. Lack of other features of MA, particularly abnormal glandular architecture and glandular cuffing aids in its distinction from an *endometrial polyp with cellular stroma*. *Endometrial polyps with bizarre stromal cell nuclei* are characterized by the presence of stromal cell atypia, most commonly in the form of multilobated or multinucleated cells, similar to those that occur in fibroepithelial stromal polyps of the distal female genital tract. These benign polyps do not show other features of adenosarcoma, particularly the intraglandular polypoid projections or periglandular cuffing by hypercellular stroma. In most cases, the atypical stromal cells are a focal finding and are not mitotically active. *Endometrial polyps with adenomyomatous stroma* can be

particularly challenging as the relatively less cellular myomatous stroma can surround the cellular, and often mitotically active, proliferative phase endometrial stromal component of the polyp mimicking periglandular hypercellular stroma. Recognition of the smooth muscle component, the lack of intraglandular polypoid projections/cleft-like glands, and the fact that the stroma is cellular throughout the area with a glandular component (rather than just tightly cuffing the epithelium) helps in this distinction. Mixed endocervical/endometrial polyps are challenging because they have glands with epithelium showing both endometrioid and mucinous differentiation, mimicking the epithelial metaplastic change that can commonly be seen in MA. In addition, areas of the polyp with endometrial type stroma can mimic periglandular stromal condensation. Awareness that mixed polyps can occur and recognition that the cellular stroma does not tightly cuff the glands is useful. In addition, these polyps should not show intraglandular polypoid projections or stromal cell atypia.

Other difficult lesions in the differential diagnosis include:

- Atypical polypoid adenomyoma
- Low grade ESS with glandular differentiation

Atypical polypoid adenomyoma is distinguished from adenocarcinoma by its uniformly fibromyomatous stroma without periglandular accentuation, squamous morular metaplasia (more common and extensive than seen in MA), and lack of cleft like glandular growth. *Low grade ESS with glandular differentiation* may occasionally contain benign glands; however, they are usually focal, lack a cleft-like appearance, and do not have cuffing by the stromal component.

There are cases in which the histologic features that typically define adenocarcinoma are subtle or focal and in these cases the diagnosis of adenocarcinoma cannot be reached. Nevertheless, the findings may be worrisome albeit quite subjective. In these cases, we advocate use of the term “endometrial polyp with atypical features” with a comment that although diagnostic features of adenocarcinoma are not identified, close clinical followup with sampling of any “recurrent” polyps is recommended.

High Grade Squamous Intraepithelial Lesion (HSIL) vs Atrophy in an Endometrial Sampling

One pitfall in diagnostic gynecologic pathology is the presence of diagnostic neoplastic tissue in a sample where it is typically not expected. While the finding of cervical tissue in an endometrial sampling is not uncommon, the finding of neoplastic cervical tissue in a sample from a patient in which the diagnostic biopsy is being performed to analyze a different tissue compartment is more surprising and easier to miss, particularly when the benign mimic (atrophy) can have morphologic overlap with the neoplastic tissue (HSIL). A spectrum of squamous epithelial changes can be associated with older age and include 1) conventional atrophic changes with immature appearing but cytologically bland squamous epithelial cells with round to oval nuclei and less abundant eosinophilic cytoplasm, 2) atrophy with partial maturation in which there is focal nuclear enlargement and 3) atrophy with maturation disturbances resulting in pseudo-koilocytosis and often resembling transitional metaplasia. While the high degree of cellularity and occasional focal nuclear atypia can be seen in atrophy, the presence of mitotic activity signifies the presence of a coexisting HSIL. In cases in which mitotic activity is not visualized but the epithelium contains uniformly hyperchromatic and crowded nuclei, the possibility of a HSIL can be excluded with special stains for p16, a surrogate marker of HPV infection and Ki-67, a proliferation marker. Atrophic squamous epithelium is negative for p16 and will have a low to absent proliferation rate in contrast to a HSIL in which the p16 will be

diffusely positive (nuclear and cytoplasmic staining) as well as show an increased proliferation rate (usually > 30%).

Metastatic Tumors to the Endometrium

The most common non-primary tumors of the endometrium originate from the cervix (adenocarcinoma), breast, and gastrointestinal tract. The characteristic features of metastatic carcinoma to the endometrium include:

- No grossly apparent lesion
- Diffusely infiltrative pattern with sparing of normal endometrial glands
- Capillary lymphatic space invasion

Three of the more difficult metastatic tumors to the endometrium to recognize in biopsy/curettage specimens are metastatic colonic adenocarcinoma, which can closely mimic primary endometrioid adenocarcinoma, metastatic lobular carcinoma, which can mimic histiocytes and metastatic serous adenocarcinoma from an upper genital site, which can mimic a primary serous carcinoma of the endometrium. Features that favor *metastatic colonic adenocarcinoma* include an abundance of acute inflammatory cells associated with necrosis in the absence of solid growth or high nuclear grade. The presence of intestinal (goblet cell) differentiation is also helpful as well as a clinical history of a prior colonic neoplasm. *Metastatic lobular carcinoma* in an endometrial sampling can easily be missed as they can mimic collections of histiocytes in an atrophic background or predecidua in a cycling endometrium. Features that help in its recognition include the more atypical appearing nuclear features, albeit subtle, in comparison to histiocytes and predecidua as well as the greater degree of cellular cohesion and “single file” growth pattern of the metastatic tumor cells. Metastatic serous carcinoma from an upper genital tract primary will typically exhibit nests of cells within cleft like spaces similar to metastatic spread in the peritoneum. The presence of papillae and hobnailed cells is typically not present. Moreover, a grossly visible lesion is lacking.

Infarcted Leiomyoma vs Leiomyosarcoma in a fragmented specimen

The diagnosis of leiomyosarcoma is based on the histologic evaluation of three parameters: mitotic activity, cytologic atypia and tumor cell necrosis. The diagnosis of leiomyosarcoma is made when any of the following conditions are satisfied: 1) diffuse or multifocal significant cytologic atypia is present and the proliferative activity is greater than or equal to 10 mitoses per 10 HPF and 2) geographic tumor necrosis is definitively present. Some consider the latter to carry the greatest weight in the determination of malignancy; however, malignancy-associated tumor necrosis has to be carefully delineated and distinguished from benign degenerative changes or other therapeutic effects (e.g. embolization, focused ultrasound). Malignant tumor cell necrosis is often multifocal, with an irregular, map- or island-like contour. There is often a sharp interface with viable cells and the necrotic foci often have atypical necrotic or “ghost” cells. A fibroblastic repair reaction at the interface is common. In contrast, benign infarct type necrosis is usually single, often central (which may be difficult if not impossible to discern in a fragmented specimen) and has an ill defined interface with a broad zone of repair reaction between the dead and viable cells. While these differing features appear relatively straightforward in theory, they are difficult to apply in practice and the reproducibility even among experienced gynecologic pathologists is fair to poor. Moreover, infarct type necrosis may be seen in leiomyosarcoma as well; therefore in a fragmented and limited sample in which only infarcted tissue is

obtained, great care should be taken in scanning the non-viable tissue for atypical nuclei. Mitotic activity can still be identified in some cases of infarcted sarcoma, which should raise one's suspicion about the underlying process. In some cases, diagnosing an infarcted smooth muscle tumor and asking for additional tissue may be useful.

Early Serous Carcinoma vs Benign Endometrium (atrophy, polyp)

Serous intraepithelial carcinoma (Serous EIC) is not a cancer precursor but an early intraepithelial malignancy that carries a risk of concomitant intra-peritoneal disease through exfoliative spread. The diagnosis of EIC should result in full surgical staging to exclude this possibility. Most cases of serous EIC are conspicuous and easily identified on routine H&E examination of the endometrium. The cardinal features include 1) involvement of surface epithelium or existing gland tracts without a change in gland density, 2) nuclear enlargement and hyperchromasia with variable stratification and exfoliation, 3) high proliferative index and 4) strong, diffuse staining for p53. More subtle examples of early serous carcinoma are more difficult to recognize because they may lack marked nuclear enlargement, which overlaps with the changes that can be seen in reactive epithelium. The differential diagnosis of early serous carcinoma includes

- Surface repair
- Exfoliation artifact
- Ischemic endometrial polyps with surface nuclear atypia
- Arias-Stella like effect produced by progestin

Surface repair typically exhibits a low nuclear-cytoplasmic ratio and tubal metaplasia with conspicuous terminal bars and cilia. *Exfoliation artifact* is a change secondary to fluid instillation into the uterine cavity, which results in mild autolysis of the lining cells, creating a hobnail appearance. This artifact lacks the nuclear enlargement and hyperchromasia of an early serous carcinoma; it also lacks diffuse staining for p53. *Ischemic endometrial polyps* often have surface epithelial atypia; however, the findings of abundant cytoplasm and underlying ischemic stromal changes including nuclear debris and hyalinization aid in its distinction. *Arias-Stella like effects* can also mimic malignancy, but lacks mitotic activity and typically shows a spectrum of nuclear abnormalities within the same gland tract, sometimes with only partial involvement.

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Non-Neoplastic Things You Can Miss If You Do Not Think Of Them.

George L. Mutter, MD

INTRODUCTION

Uncommon presentations of common diseases, fragmented specimens, sampling error, and otherwise suboptimal specimens are only a few of the many sources of diagnostic difficulty in endometrial pathology. Combined with a "normal" reference point which changes dynamically throughout the month, and during the life cycle, the very definition of "abnormal" depends on the clinical setting. This section will review diagnosis of several benign but clinically significant conditions which are easily missed, presenting common differential diagnoses and clinical implications of its resolution. Many of the described entities are illustrated in common subspecialty textbooks such as Robboy's Pathology of the Female Reproductive Tract. 2 ed. New York, New York: Elsevier, 2009.

PREGNANCY PAST AND PRESENT.

Fibrohyaline nodules are expansile cerebriform areas of dense extracellular matrix that form at the site of implantation. During ongoing pregnancy, well preserved trophoblastic elements may be seen, but these resorb slowly following delivery, leaving behind the nodular structure which may persist for years thereafter. In practice, presence or absence of trophoblast, and its degree of preservation, varies greatly amongst examples of fibrohyaline nodules, and it is often impossible to determine whether the responsible gestation is current, or years in the past. In the endometrium with FHNs, other landmarks of ongoing or recent pregnancy such as implantation site, villi, and stromal decidualization may be informative, but an isolated FHN may be recent or quite old.

Clinical Tip: Beware dating of FHN's, but if found look for other signs of ongoing pregnancy.

Pseudodecidua (exogenous hormones) vs Decidua Vera (pregnant) vs Predecidua (cycling). The terminology used to describe stromal changes of progestins has substantial clinical impact. Predecidua refers to normal endometrial changes in the luteal phase of a normal cycle, decidua (vera) refers to endometrial stromal changes in the pregnant patient, and pseudodecidua refers to either pharmacologically induced changes in native endometrium or stromal decidual changes at sites other than the endometrium itself. These very different entities, all characterized by stromal decidualization, must be clearly resolved and communicated. Predecidua in the cycling patient tends not to occur in large sheets, has characteristic cycling glands. Upon menstruation following a normal cycle the predecidual cell cytoplasm contracts and nuclei collapse into basophilic aggregates "blue balls", in contrast to the uncollapsed eosinophilic expanses of necrotic pseudodecidua and decidua vera.

Clinical tip: If necrotic decidualized stroma is pale eosinophilic with uncollapsed "ghosts" consider the possibility of pregnancy or prior hormonal therapy.

Retained products in the form of implantation site, villi, and/or membranes may be seen weeks after delivery. Abnormal implantation or gestational trophoblastic disease needs to be excluded. Special stains are rarely necessary for detection of retained products, but when desired, pankeratin stains are positive in trophoblast while negative in endometrial stromal cells. Background endometrium may be

reparative, cycling, or non-cycling. Endometritis occurs in 2–5% of women following vaginal delivery, 20–55% of women following cesarean section and 1–8% of women having termination of pregnancy in a hospital setting. The risk of endometritis is even higher in women with retained products of conception

Clinical Tip: Retained products may be accompanied by a variety of background endometrial patterns, but chronic endometritis should always be specifically ruled out.

“Floaters” are laboratory contaminants between cases, and the friable nature of placental tissue explains frequent occurrence of displaced placental villi between specimens. This can be particularly disconcerting in the rule-out ectopic endometrial sample. Most rule-out ectopic scenarios occur early in the first trimester, while smaller mature term villi are most common floaters.

Clinical Tip: Beware when villous maturity is discordant with clinical gestational age.

UTERINE PERFORATION

Endometrial curettages may contain unexpected (non-fetal, adult) normal tissue types such as fat or bowel. These can represent a contaminant (floater) or sampling of extrauterine tissues at time of perforation. Identification of fat is sometimes difficult, as a regular pattern of air bubbles in fixed proteinaceous slurry is a close mimic. In our experience most perforations are identified histologically, without a pre-existing story in the pathology requisition. Thus, the clinical history is usually noncontributory and it is the pathologist who informs the clinician that perforation has occurred.

Clinical Tip: When extrauterine tissue is found in an endometrial sample, consider perforation

NON-ENDOMETRIAL FINDINGS

Endometrial sampling devices must transit the vagina, cervix, and lower uterine segment, any of which may contribute tissue to the final specimen. Misidentification of tissue type, or failure to recognize pathologic processes in sampled non-endometrial tissues may have clinical implications.

Squamous Epithelium is never normal in the endometrium, but strips of cervical squamous epithelium or aggregates of vaginal squamous cells are common in endometrial biopsies. Squamous morules, concentric round masses of squamous cells, are usually of endometrial origin and have a statistical association with endometrial neoplasia.

Clinical Tip:

-If non-endometrial squamous tissue is present, state the origin (e.g., fragments of benign endocervix with squamous metaplasia)

-If morules are present, diagnose as such and look carefully at endometrial glands.

Mucinous epithelium: Normal endocervix is present in the majority of endometrial samples, but mucinous epithelium is never normal within the endometrium itself. When present as detached strips, endocervix from the t-zone will have a lower layer of reserve cells rarely seen in endometrial mucinous differentiation. Excessively abundant mucinous epithelium, delicately arborized and redundant mucinous folds, or alternating mucinous and non-mucinous epithelium, should raise the

possibility of an endometrial lesion with mucinous differentiation.

Clinical tip: Look carefully at detached mucinous epithelium for signs of endometrial origin

ENDOMETRITIS

can be subtle or fulminating, and may initiate therapy or a search for an underlying cause.

Chronic endometritis is classically diagnosed by recognition of plasma cells within native endometrial functionalis on routine H&E stain. While these can only be identified with certainty on high magnification, there are usually clues at low power which should initiate a search. Endometria with intraluminal neutrophils, uneven or patchy lymphocytic infiltrates, or dys-synchronous cycling should always trigger a screen for plasma cells.

Clinical Tip: Look for plasma cells if something is remiss at low power.

Pyometrium is a severe form of acute endometritis that is easy to diagnose. It is almost always accompanied by a predisposing condition such as cervical incompetence, fistulous tracts, foreign bodies, or necrotizing or perforating tumor.

Clinical tip:

- Try to identify an underlying cause in cases of pyometrium.
- Polarized examination may help to identify stool from utero-colic fistulas.

HORMONAL CONDITIONS

If a sample is technically adequate and clinically consistent with an appropriate cycling, inactive, or atrophic histology, diagnosis is straightforward. Not so when the findings are non-physiologic or there is a discrepancy between histology and clinical context.

The delayed ovulatory “anovulatory” patient We often think of estrogen induced endometrial changes of irregular cysts, fibrin thrombi, and irregular breakdown as “anovulatory,” forgetting that delayed ovulation carries forward all of these features to a postovulatory environment with secretory change. There are no consistent descriptive terms which describe this functional state of delayed ovulation.

Clinical tip. Delayed ovulation is common, and better diagnosed using functional (delayed ovulation) rather than descriptive histologic (cysts, thrombi, breakdown) language.

Exogenous hormonal changes may be difficult to specifically infer from the histology alone. The days of simple formulation high dose oral contraceptives that yield consistent and recognizable endometrial changes are gone, replaced by multiple agents and regimens that sometimes yield a morphologically normal or uniquely altered endometrium. The pathologist should develop a clear notion of what can be definitively concluded from the histology, compared to what is inferred by reference to the clinical history. Always use caution in carrying forward overriding clinical diagnoses that cannot be directly substantiated by the histology itself.

Clinical tip:

- Be prepared for an incomplete or incorrect clinical history.
- Distinguish between findings that are internally diagnostic and those consistent with, but not diagnostic of, a clinical state.

- Recognize that synthetic compounds may create bizarre constellations of changes outside what we understand from a simple progesterone-estrogen model.

When inadequate is enough Postmenopausal patients just do not have a lot of endometrial tissue, with poor yields even in the hands of experienced clinicians. Unfortunately, the postmenopausal patient is frequently biopsied, and demographically at high risk for diseases which increase in incidence with age (like cancer). The scanty sample in the postmenopausal patient creates a dilemma for the pathologist who struggles to balance accurate reporting of the skimpy specimen with qualifications of inadequacy. Recall, however, that the elderly patient with endometrial cancer or EIN will have a bulkier tissue volume more amenable to sampling. It is not helpful to the clinician submitting a scanty specimen to receive a report stating an unqualified “inadequate for diagnosis.” Describe what, if any, tissue is present (blood, endocervix, surface endometrium). This will allow the clinician to know which cavities they actually sampled, and fulfill reporting requirements for reimbursement of histologic processing and examination.

Clinical tip:

- If no tissue is present, say so.
- Even if scanty, describe what is present microscopically.
- Consider descriptive diagnoses such as “Scant fragments of inactive surface endometrium”

ENDOMETRIAL POLYPS are typically removed piecemeal, and fragments interspersed with other tissues in the endometrial biopsy. Especially when the background endometrium is atrophic (scanty), it is not always clear whether the observed fragments represent a diffuse or localized process. Polyps are sufficiently common that they frequently coexist with other pathology.

Clinical Tip:

- Look carefully for background non-polyp fragments and diagnose it separately
- Remember that polyps can coexist with other lesions (like EIN) within the polyp which must be separately diagnosed.
- Polyps should not be used to infer the patients hormonal state. They do not cycle.
- Endometrial polyp is a histologic, not a gross (or hysteroscopic) diagnosis

ARTIFACTS

Cautery, crush, and poor fixation will distort tissue in recognizable ways familiar to most pathologists. More subtle are alterations caused by non-isotonic immersion during hysteroscopy or accidental collection in water. Surface exfoliation caused by instillation of non-electrolyte fluids (like glycine) during hysteroscopy can mimic serous neoplasia.

Clinical Tip:

- Be familiar with “high tech” artifacts introduced at hysteroscopy
- If a specimen cannot be diagnosed because of artifacts, make this clear in the report and consider a recommendation for rebiopsy.

DIAGNOSIS BY INFERENCE

Myometrial lesions are poorly sampled by endometrial biopsy or curettage, yet may be suspected based upon the histologic findings. An example is focally aglandular surface endometrium overlying an (unsampled) submucous leiomyoma.

Clinical tip: If an inferred lesion is suspected, make a “consistent with” diagnosis.
e.g. strips of aglandular endometrium consistent with submucous leiomyoma,
fragments of hypertrophic smooth muscle consistent with leiomyoma.

Challenging Cases from Daily Practice.

Drs. Nucci and Mutter

In this final session Drs. Mutter and Nucci will present difficult and illustrative cases using virtual microscopy.