

Equine endometrial biopsy: Enhancement of clinical value by more extensive histopathology and application of new diagnostic techniques?

D.H. Schlafer*

T-020 Veterinary Research Tower, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, United States

Abstract

During the 1960s and 1970s, the clinical value of equine endometrial histopathology was firmly established after it was shown that fertility outcome was correlated with the presence and severity of specific microscopic lesions. The objective of this paper is to summarize reports from the veterinary literature published after the mid 1980s that describe new diagnostic methods of assessing equine uterine health using material collected by endometrial biopsy.

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1. Introduction

Assessment of the health status of a mare's uterus by endometrial biopsy and histopathology has been a standard procedure in equine reproduction for >35 years. In the last two decades, many new diagnostic techniques have been developed, yet histopathology based on hematoxylin and eosin stained tissue sections remains the primary method of endometrial assessment [1,2].

This paper explores ways that endometrial tissues have been studied in recent years. The objective is to generate discussion of how new techniques and research findings can be used to provide more information of direct clinical use.

Many techniques have been used to study the equine endometrium (Table 1). New research methods and equipment have enabled researchers to generate

extensive quantitative data (Table 2). Although the clinical usefulness of some procedures are technically complex or too costly for practical inclusion in routine testing regimes, in the future, biopsy material from valuable mares may undergo more extensive testing.

1.1. Endometrial biopsy of the mare: historical perspective

Equine endometrial biopsy was first used clinically in the 1960s after suitable instruments became available and the description of primary endometrial lesions was published [1]. In 1969, Drs. Brandt and Manning set the stage for future research regarding endometrial biopsy by stating the goals for their own work: "Technics are needed that will increase the practitioner's potential for more accurately evaluating the functional status of the equine uterus. Only when more direct technics have been perfected can *in vivo* studies lead to greater understanding of the equine uterus in health and disease. It is hoped that uterine biopsy may prove to be such a technic."

* Tel.: +1 607 253 3352; fax: +1 607 253 3541.

E-mail address: dhs2@cornell.edu.

Table 1

Experimental techniques used in the assessment of the anatomy, physiology and pathology of the equine endometrium since the mid 1980s

Parameter	Method	Comment	Reference	Year
Histopathology	Cytochemistry			
	-Alcian blue	Extracellular matrix	[34]	2001
	-PAS	Blood vessels, mineral in glandular	[55]	2001
	-Van Kossa	Concretion	[47]	2003
	-Trichrome	Fibroplasia	[56]	1987
	-Picrosirius Red	Collagen	[50]	2006
	Lectin histochemistry	Binding patterns	[34]	2001
	Immunohistochemistry			
	-Immunoglobulins	Distribution	[27]	1991
	-Monocytes and macrophages		[17]	1998
	-Cell proliferation	Ki-67 protein	[12]	2000
	-Proteins	Endometrial proteins	[46]	2003
			[47]	2003
	-Matrix proteins	Osteopontin, osteonectin	[51]	2005
	-Metalloproteins and tissue transglutamine	Collagen		
	-Mast cells		[22]	1997
	-Intermediate filaments	Vimentin, cytokeratin, desmin	[48]	2004
	-T-lymphocytes	Endometrial distribution	[44]	2000
	-Hormone receptors	Estrogen and progesterone	[12]	2000
	-Hormone	Oxytocin	[45]	2003
	-Smooth muscle	Smooth muscle actin	[46]	2003
	-Laminin	Basement membrane	[46]	2003
Functional assay	Neutrophil chemiluminescence	Neutrophil function	[57]	1991
	Reactive oxygen generation	Neutrophil function	[21]	2004
Ultrastructural microscopy	Transmission electron microscopy	Endometrial epithelia	[58]	1995
		Fibrosis	[49]	1998
		Fibrosis (myofibroblasts)	[50]	2006
			[26]	1999
	TEM-immune labeling	Oxytocin	[45]	2003
		Vimentin	[48]	2004
	Scanning electron microscopy	Pathology	[39]	2004
<i>In situ</i> hybridization		Epidermal growth factor	[35]	1999
Biochemistry	SDS page gel electrophoresis	Protein analysis	[30]	1998
	DNA and RNA	Nucleic acid quantification	[55]	2001
Bacterial adhesion	<i>In vitro</i> culture	Bacterial adhesion	[59]	2000
Real-time PCR		Cytokines IL-1beta, IL-G, TNF-alpha	[43]	2003
Flow cytometry		Cellular phagocytosis	[21]	2004
Gelatin zymography		Matrix metalloproteinase	[51]	2005
ELISA		Antigen capture TGF-b	[50]	2006
Autoradiography			[50]	2006
Radiometric assay			[50]	2006

A series of publications followed that described improvements in collection techniques, further elucidated key microscopic changes (lesions), and demonstrated clinical relevance through correlation of results of endometrial histopathology with fertility as assessed by pregnancy outcome [2–6]. A review published in 1988 provided a detailed account of progress up to the mid 1980s [7].

1.1.1. “Classification” schemes and endometrial histopathology from the 1970s and 1980s

During the 1970s and 1980s, criteria for endometrial histopathology, based on light microscopic examination of endometrial tissues, sectioned and stained by hematoxylin and eosin stains, were established. Although different classification “schemes” were developed, the system most widely used today is that

Table 2
Studies using quantitative measures to assess anatomy, physiology and pathology of the equine endometrium

Parameter	Reference	Year
Inflammatory cell populations	[22]	1997
Isotypes of plasma cells	[27]	1991
Uterine arteries	[52]	1995
Glandular structures	[29]	1996
Amount of fibrosis	[49]	1998
Endometrial macrophages	[17]	1998
Steroid hormone receptors	[12]	2000
Proliferating cells	[12]	2000
Endometrial microvasculature	[55]	2001
DNA and RNA quantification	[55]	2001
Phagocytosis by neutrophils	[21]	2004
Endometrial growth factors	[50]	2006

published by Drs. Kenney and Doig in 1986 [2–4,8]. This classification system evolved from classic works of descriptive pathology by Drs. Kenney, Ricketts, Doig and others [2,8,9,10]. At least two other classification are in use today [5,11]. These classification systems are based on identification of histologic features associated with degree of acute or chronic inflammation, endometrial fibrosis, or endometrial atrophy (or hypoplasia). These results are combined with clinical information to arrive at an interpretation of the clinical relevancy of the findings. The “modified” Kenney Doig system published in 1986 uses four categories (1, 2A, 2B, and 3) to summarize the health status of the endometrium based on all information made available. Many clinical trials have confirmed surprisingly strong correlation between endometrial lesions and the ability of the mare to carry a foal to term the following year.

2. Endometrial histopathology

Many excellent reviews have been published that deal with various aspects of endometrial changes associated with season and age [5,12,13–17,18], inflammation [2,19,20], host defenses [21,22], and pathology [2,4,5,11,15,23–27]. It is beyond the scope of this paper to include all published reports and the interested reader is referred to the reference list as an entry point into the literature.

2.1. Limitations of endometrial histopathology

Although the type of information that can be extracted from histopathology is limited, it is likely that histopathology will remain a cornerstone in the assessment of endometrial health. The standard procedures involve recognition of changes by conven-

tional light microscopy in the fixed tissues sectioned and stained with hematoxylin and eosin stains. Factors that negatively impact the value of endometrial biopsy include poor quality of submitted material, failure of provision of critical pertinent clinical information, the level of experience or interest by diagnostic or clinical personal involved, and lack of assessment of diagnostic findings in terms of clinical relevance. An important aspect of the historical development of equine endometrial biopsy assessment systems is that those individuals who did the pioneering work had strong backgrounds in both pathology and equine reproduction. The key players were, therefore, in the unique position of being able to evaluate microscopic changes they identified in sections of endometrium in the context of the clinical status and subsequent reproductive performance of the individual mare from which the biopsy was derived. Today many clinicians who are not primarily working with brood mares, rely on general diagnostic pathologists in state or private laboratories for interpretation of endometrial histopathology. It difficult for most clinicians or general diagnostic pathologists to become proficient in biopsy interpretation unless they have a sufficiently high case load. Although unavoidable, this limits the usefulness of endometrial biopsy as a clinical tool and argues for establishment of more specialized laboratories.

As will be discussed, some newer diagnostic procedures have potential for high “through-put” and can be automated. The ability to generate quantitative data may remove some subjective aspects inherent in histopathology. Examples of techniques that have been used and hold promise for use in the future include computer based tissue morphometry, RT-PCR, ELISA, and genetic array analysis. The need for specialized equipment and expertise limits access to these complex diagnostic tools, yet their use in specialized laboratories will steadily increase in the future. As many of these newer assay systems require only very small amounts of tissue, it is likely that single endometrial biopsies will be able to be used for many different types of testing.

2.2. Endometrial biology, histology and pathology

The uterine wall can be viewed as containing seven inter-related components. There are corresponding functions that can be associated with each one.

- 1 Uterine lumen
- 2 Endometrial epithelial interface
- 3 Superficial stroma (stratum compactum)
- 4 Glands and glandular epithelium

- 5 Stroma—mid and deep endometrium
- 6 Vessels (arteries, veins and lymphatics)
- 7 Myometrium and larger vessels

The discussion to follow is organized using this framework as it provides a way to conceptually consider the elements that make up the uterine wall and its lumen and is helpful in consideration of the patho-physiology of uterine disease.

2.2.1. Uterine lumen and the endometrial epithelium

The luminal epithelium is the “functional interface” between the uterus and the outside world and once pregnancy is established, the luminal epithelium is directly attached by microvilli to placental trophoblast cells (epithelialchorial placentation).

Endometritis is the most important endometrial lesion. Introduction of microbial agents, urine or air into the uterus through the cervix occurs much more commonly in the mare than in other domestic species. It has been shown that vulvar conformation has a significant effect on pregnancy and live foal rates [28].

Endometrial histopathology has heavily relied on subjective assessment of the nature and severity of tissue changes. Use of techniques that provide quantitative data, such as tissue morphometrics, offer substantial promise [29]. An example of this is the work by Rasch et al. [29] who used a semiautomated microscopic analysis system to obtain quantitative measurements of the height and width of the surface epithelium, the area of the endometrium occupied by endometrial glands, the gland density, and the maximal size (diameter) of endometrial glands. They compared results of analysis of tissues collected from 49 mares with intrauterine fluid and 10 control mares and found that mares with fluid had significantly higher gland density and larger glands (diameters and size of their lumen), and used those data as evidence that the pathogenesis of excessive fluid accumulation may include excessive production. They concluded that morphometry was “a sensitive diagnostic aid” for study of endometrial disorders.

Biochemistry has also been used to study uterine secretions. The chemical composition of uterine fluids has also been characterized by precise protein analysis systems (SDS-PAGE) and quantitative immunology (immunoglobulins IgA and G) [30]. In a study involving 20 normal mares, greater variation in protein concentrations was found to occur in uterine fluids than that found in serum proteins among mares. It was concluded that there was “considerable” transudation into the

uterine cavity [30]. This leads to the question whether an inflamed endometrium would not “leak” more and higher molecular weight proteins (forming an exudate) and if so, could rapid quantitative assays be used to identify endometritis by uterine fluid protein determination.

As one would expect, host defense systems have received considerable attention. Transferrin, functions in defense by withholding iron and has an important role in control of bacterial infections. Mares with different transferrin genotypes were shown to have differences in severity of endometrial lesions [31]. Other work has focused on the role of inflammatory cells in control of bacterial infections [19,21,22,24,27,32,33].

Interpretation of microscopic changes within the endometrium has always been complicated by alterations in endometrial histology that are associated with season and stage of cycle. Immunohistochemical techniques have made it possible to study changes in specific hormone receptors, and cellular proliferation from formalin-fixed endometrial samples [12]. Another technique, lectin histochemistry, was used to study changes in lectin staining patterns of secretory epithelia cells; there were indications that glycoconjugate patterns in mares with chronic endometrial degeneration may reflect functional impairment of the epithelial cells [34]. It is feasible that these specialized staining procedures could be used in the future to more accurately assess cyclical status or identify abnormal epithelial cells.

Application of molecular techniques for assessment of gene expression has become routine in biomedical research. It is surprising that more work has not been done with the equine endometrium. These techniques use labeled short segments of nucleic acid to attach to matching sequences contained in cellular nucleic acids. Hybridized probes firmly attach to corresponding alternative base pair sequences. Non-bound labeled probes are removed by washing and bound probes can be detected using various techniques. Advantages associated with the use of molecular probe hybridization procedures include exquisite sensitivity, specificity, the testing procedures can be automated, and the tests require very small samples. Activity of specific genes within a cell can be qualitatively and quantitatively assessed. *In situ* hybridization is a molecular based staining procedure that can be done on sections of tissue. Specific nucleic acid sequences can be localization to specific cells, cellular structures, microorganisms, etc. *In situ* hybridization has been used to monitor levels of the growth factor EGF mRNA in equine endometrial tissues collected by biopsy [35]. In this

study, the authors examined endometrial tissues collected from seasonally anestrous mares, ovariectomized mares given progesterone and estrogen, and pregnant mares with either normal and or abnormal endometrium. They reported that endometrial glands compromised due to inflammation or fibrotic change had an absence of EGF mRNA.

Controlled experimental studies that model uterine disease have involved infusion of compounds or organisms directly into the uterine lumen [36–39]. One study used a compound (an inflammatory mediator) that had been produced using recombinant molecular procedures [21].

Degenerative changes have been described in endometrial epithelial cells from mares with mycotic infections [40]. Other studies have examined epithelial cells in abnormal endometrial tissues by transmission electron microscopy [26].

2.2.2. Superficial interstitium (*stratum compactum*)

The superficial stroma is highly vascularized and is therefore an important site of exocytosis and accumulation of inflammatory cells. The surface layers of the endometrium also undergo dramatic remodeling in pregnancy. The repair and remodeling processes associated with uterine involution are complex, but amazingly efficient [42]. Changes in cell populations during involution have been studied quantitatively using immunohistochemistry for identification of specific cell types in tissues [22].

Because infection stimulates a local inflammatory reaction that can lead to permanent damage to the endometrium, there has been interest in understanding the inflammatory reaction as it affects all levels of the endometrium. Production and response to mediators of inflammation have been assessed using molecular techniques. The mRNA expression of the pro-inflammatory cytokines IL-1beta, IL-6 and TNF-alpha within the endometrium during the estrous cycle, after insemination, and after inoculation of the uterus with bacteria, have been studied [43]; mares classified as resistant had lower basal levels of genes for these pro-inflammatory cytokines than mares characterized as susceptible.

The role of lymphocytes in the local uterine immune response has been studied extensively, and includes quantification of cellular infiltrate following experimental exposure to a variety of material. The T-cell (CD4 and CD8) response to inflammation after insemination was evaluated using immunohistochemistry at two time points [44]; a CD4 helper T-cell response was detected 6 h post-insemination.

Macrophage numbers and distribution during the estrous cycle of genitally normal mares and mares susceptible to persistent endometritis have been studied using monoclonal antibody labeling by immunohistochemistry [17]. Surprisingly, no changes were found, based on cycle stage or reproductive status.

2.2.3. The glandular epithelium

Combined with the luminal epithelium, the glandular epithelium serves to produce and release complex biological secretions (uterine fluid, uterine milk, histotroph) that vary depending on stage of cycle and pregnancy status. Physical damaged or functional asynchrony of epithelial cells is associated with subfertility.

Secretory function of the epithelium has been studied. Uterine fluid consists of secretory products from epithelial cells lining the epithelial lumen, cells lining the endometrial glands and transudates leaking through the endometrium. The secretory epithelium has been studied for its hormone receptors and cellular proliferation (ki-67) [12]. Electron microscopy using immunogold labeling has detected oxytocin in secretory granules [45] and lectin staining has revealed alterations in staining in tissues from mares with chronic endometrial degeneration [34].

Endometrial glandular secretions and possible disturbances in the uterine microenvironment were evaluated by immunohistochemistry (specific for uteroglobulin, calbindin, uteroferrin, uterocalin and glycogen) on endometrial tissues collected from 620 mares [46]. Staining patterns of endometrial secretions from normal glands exhibited cyclic patterns, whereas there was distinct loss of staining intensity in glands with endometrial fibrosis [46]. Immunohistochemistry has also been used to study the composition of mineralized concretions contained in glandular lumen of mares [47]. Using immunohistochemical methods, the non-collagenous matrix proteins osteopontin, osteonectin and bone sialoprotein were found. These proteins are associated with calcification. The authors studied biopsies from 23 mares and found that these mineralized structures occurred commonly in endometrial containing chronic degeneration [47].

2.2.4. Mid and deep stroma—*stratum spongiosum*

The endometrial stroma that supports the parenchymal elements (glands and vessels) of the middle to deep levels of the endometrium is often associated with fibrotic changes that may impact glandular function and fertility. Abnormalities in fibrotic stroma and within entrapped glands have been demonstrated. Abnormal

expression of cytokeratin and vimentin has also been demonstrated in abnormal endometrial stromal tissues [48]. Steroid receptors on stromal cells have identified asynchrony of cells within areas of periglandular fibrosis [12]. Epithelial cells lining glands in affected areas were abnormal. Endometrial glands compromised due to inflammation or fibrotic change had an absence of EGF mRNA [35] and there was specific loss of immunohistochemical staining intensity for a variety of secretory proteins in glands within areas of endometrial fibrosis [46].

Quantitative assessment of the amount of endometrial fibrosis can be assessed by use of stains specific for collagen combined with computer based image analysis [49,50]. Studies have reported on the roles of matrix metalloproteinases and growth factors (including TGF-beta1) in the development of endometrial fibrosis [50,51].

2.2.5. Blood vessels and lymphatics

Various sizes of arteries, veins, capillaries, and lymphatic vessels carry blood to and from the endometrium and drain lymph away from the endometrium. There is an extensive network of capillaries that form a fine network just beneath the luminal epithelium. Age-related changes in the walls of uterine arteries are common [52,53]. Vascular degenerative changes associated with aging (wear and tear) must be differentiated from vascular lesions caused by a specific insult. Until recently, there has been relatively little research in this area. Lesions of vessel wall had been reported following experimental infusion of iodine [38] and arteritis caused by EVA [54]. Nambo et al. studied changes in the walls of uterine arteries in tissues from 13 mares of varying ages. They found hyperplasia of smooth muscle cells in the tunica media and an increase in elastic fibers that were directly associated with age [52]. Similar finding were reported from a larger study involving histopathology of 117 endometrial biopsies from mares of varying age and known reproductive status [53]. “Inflammatory vascular alterations” were identified in slightly over 20% of the tissues examined; no vascular lesions were found in maiden mares. The incidence as well as the severity of vascular lesions increased with the number of pregnancies and with aging. Lesions of the vascular wall, fragmentation of the elastic fibers, activation of smooth muscle cells, and the presence of immature elastic fibers in the intima and inner media were reported to be similar to “pregnancy sclerosis” of other species [53].

Endometrial angiogenesis during the estrous cycle has been studied by assessing the density of tissues

stained by periodic acid-schiff and evaluated using a computer image analysis system [55]. There was no difference between mares in the follicular versus luteal phase of their cycles. It would be interesting to use quantitative techniques such as this to study vascular changes in mares with experimentally induced endometritis.

2.2.6. Myometrium

The myometrium consists of two layers of smooth muscle, nerves, larger arteries, veins, lymphatics and stomal elements, covered by a thin serosa. Only small amounts of myometrial tissue are usually present in biopsies, but its function is important in uterine clearance, in provision of an adequate blood supply, and efficient drainage of lymph and blood.

3. Methods used to assess endometrial tissues

Cytochemical and immunohistochemical staining are routine procedures in veterinary diagnostic laboratories, and as such are prime candidates for assessing endometrial biopsies. Trichrome histochemical stain is widely used for detecting endometrial fibrosis [56]. Use of other cytochemical stains have been reported (Table 1) [34,47,50,55]. Of these, one of the most promising is Picrosirius Red, as it specifically stains collagen. It has been used in conjunction with computer-assisted quantitative analysis systems to evaluate fibrosis [49,50]. Interstitial endometrial fibrosis is one of the most prominent lesions associated with chronic endometrial degeneration. Although significant endometrial fibrosis is readily apparent in H and E stained tissue sections, a simple method for quantifying collagen deposited within the endometrium would be valuable.

Immunohistochemistry (IHC) is commonly employed in biomedical research and diagnostics. Antibodies against a wide variety of cellular, and stromal antigens have been used in many different IHC systems to study equine endometrial morphology, function and disease [12,17,22,27,44–48,51].

The role of antibodies in the host immune response has been studied extensively [24,27,57]. The presence of antibodies actively secreted into the uterine lumen, or that enter uterine fluid as a result of significant inflammation could potentially be used to indicate the presence of a host reaction to specific microbial infections [27]. Using ELISA procedures, it has been shown that antibodies directed against epitopes on specific microbial agents can be detected in uterine fluids [57]. Perhaps simple screening procedures can be

developed, using either ELISA or molecular diagnostic systems to screen endometrial biopsy samples for uterine pathogens. Bacterial culture and cytology taken directly from biopsied tissue have proven to be more accurate than those taken from uterine swabs [41].

Ultrastructural studies have addressed normal endometrial anatomy [58] and pathology (primarily endometrial fibrosis) [26,49,50,53] using either transmission or scanning electron microscopy. Electron microscopy will likely continue to be limited to research applications.

Growth factors in endometrial tissues have been studied using *in situ* hybridization [35], ELISA [50], autoradiography [50], and radiometric assay [50]. Gelatin zymology was used to study the role of matrix metalloproteinase [51] in endometrial fibrosis. Uterine proteins and nucleic acids have been evaluated biochemically [30,55], and pro-inflammatory cytokines in the endometrium have been identified and changes analyzed by rt-PCR.

3.1. Techniques that yield quantitative data

There are great advantages to assay systems that produce quantitative data. Examples of methods that have generated valuable quantitative data are shown (Table 2). These studies have provided information about individual cell populations within the endometrium [17,21,22,27,29], aspects of endometrial morphology and pathology [29,52,49,50,55], changes associated with stage of the estrous cycle [12] and host defense [17,21,22,27].

3.2. *In vivo* studies of the equine uterus

Interpretation of changes contained within endometrial tissue requires an understanding of how endometrial tissues respond to various insults. A sense of the breadth and depth of research that has been done over the last 20 years can be gained by considering the tests and analytic procedures listed in Table 1. *In vivo* experimental studies addressing endometrial inflammation, bacterial endometritis, mucosal immunity, and endometrial pathology are listed in Table 3 [62–67].

4. Expanded testing of biopsied endometrial tissue

Additional information can be acquired by expanded use of cytochemical and immunohistochemical stains. Diagnostic histology laboratories routinely provide these services and the use of automatic staining systems

Table 3

Experimental perturbations of the equine uterus involving direct instillation of compounds or microbial agents into the uterine lumen (selected examples taken from the literature published since the mid 1980s)

Perturbation	Reference	Year
Air	[60]	1984
Indwelling catheter	[40]	1986
Bacteria		
- <i>Taylorella</i>	[61]	1986
	[62]	2000
	[57]	1991
- <i>Streptococcus</i>	[24]	1993
- <i>Pseudomonas</i>	[63]	1992
Microspheres (radiolabeled)	[33]	1989
Human albumin (radiolabeled)	[33]	1989
Charcoal	[33]	1989
Dimethyl sulfoxide	[36]	1988
	[37]	1988
	[64]	1989
Oyster glycogen	[65]	1990
	[66]	1994
Sperm	[16]	2005
EVA (equine viral arteritis)	[54]	1996
Gentamicin	[39]	2004
rhIL-8 (human recombinant interleukin 8)	[21]	2004

makes the cost relatively modest. Results of studies have suggested that the presence of mediators of inflammation, specific growth factors, and patterns of intermediate filaments may help characterize endometrial lesions. Incorporation of tissue morphometrics, ELISA, or molecular diagnostic procedures described above is currently limited to research laboratories, but in the future, access to selective testing will likely become available through specialized laboratories.

Detection of immune responses of individual mares to specific pathogens may be possible in the future. Uterine fluids have been shown to contain antibodies directed specifically against bacteria to which they were exposed (*Streptococcus zooepidemicus* and *Taylorella equigenitalis*) [57,61]. This work suggests that screening panels could be developed for detecting specific uterine antibodies produced in response to infection by common bacteria, fungal agents and viruses.

Most diagnostic laboratories have incorporated molecular diagnostic testing in their routine work. Great advances have been made in identification of microbial agents using molecular techniques (i.e. *in situ* hybridization, fluorescent *in situ* hybridization (FISH), genetic arrays, etc.). Bacteria can be identified in tissue sections by FISH [68] through use of appropriate probes

(labeled sequences), but these techniques have not yet been used with biopsy material.

Additional tests based on molecular techniques include identification of viruses and fungi, detection and quantification of inflammatory mediators (cytokines) or growth factors associated with endometrial fibrosis. With further research, it will be possible to detect changes in hormone receptors and changes in cellular expression of different genes that can be used to confirm stage of the estrous cyclic or seasonal state. “Up- or down-regulation” of specific genes is routinely being studied in other tissues using automated genetic array analysis systems. These techniques provide information about the status of many aspects of cellular and tissue function and are powerful tools in understanding cellular function.

5. Conclusion

A brief overview has been presented that summarizes the breadth and nature of studies on equine endometrial biology and disease since the mid 1980s. A primary objective was to identify potential diagnostic methods that were clinically relevant as many of the current research techniques are not practical. Although classic histopathology will likely remain a central tool, using additional histochemical and immunohistochemical procedures would provide valuable information. Introduction of molecular based testing, such as FISH, RT-PCR, genetic arrays analysis, etc. is inevitable and will undoubtedly provide detailed information about the biology and health status of a mare’s uterus based on analysis of biopsied endometrial tissues beyond which we can imagine possible. Although “some subjective opinion is unavoidable” [5], the introduction of quantitative testing procedures would provide greater accuracy in interpretation of findings. In practical terms, the value of endometrial biopsy would be enhanced through its expanded use. Establishment of specific laboratories would enable more complex testing and increased rigor in endometrial histopathology.

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