Endometrial Biopsy in Holstein-Friesian Dairy Cows II. Correlations between Histological Criteria

Brenda N. Bonnett, Richard B. Miller, S. Wayne Martin, Wayne G. Etherington and Brian C. Buckrell

ABSTRACT

Endometrial biopsies were taken for histological assessment from 97 cows which calved in a commercial dairy herd between April and August 1984. The main objectives of this study were to analyze the interrelationships among histological criteria and to identify a shortlist of histological parameters to be included in subsequent analysis of associations with results of bacteriological culture, clinical findings and reproductive performance. Epithelial height and segmented cell counts were highly correlated within biopsy, between horns and between days. Subjective assessment of inflammation in the epithelium and/or stratum compactum generally identified biopsies which had any inflammation present. Cows which had inflammation in a biopsy from day 26 were likely to show inflammatory changes at day 40. Quantitative and subjective assessments of gland number, dilation and fibrosis were highly correlated. There was a positive association between the number of cross sections and the diameter of glands, and both of these criteria were negatively correlated with fibrosis and inflammatory changes. There may be different functional significance of the same histological finding at a different number of days postpartum.

RÉSUMÉ

Des biopsies de l'endomètre furent prélevées après le vêlage chez 97 vaches d'un troupeau laitier commercial entre les mois d'avril et d'août 1984 afin d'évaluer leur statut histologique. Les objectifs principaux de cette étude visaient à analyser les interrelations entre les critères histologiques et en identifier une courte liste, en relation avec les résultats des cultures bactériologiques, des constatations cliniques et de la perfomance reproductrice. La hauteur de l'épithélium et le nombre de cellules segmentées étaient hautement corrélés à l'intérieur des biopsies entre les cornes et entre les jours. L'évaluation subjective de l'inflammation de l'épithélium et/ou du stratum compactum identifiait généralement les biopsies qui présentaient une inflammation. Les vaches qui présentaient une réaction inflammatoire dans la biopsie obtenue au jour 26 démontraient aussi des changements inflammatoires au jour 40. Les évaluations quantitative et subjective du nombre, de la dilatation et de la fibrose des glandes étaient aussi hautement corrélées. Il y avait une association positive entre le nombre de coupes transversales et le diamètre des glandes et ces deux critères étaient négativement corrélés avec la fibrose et les changements inflammatoires. Une signification fonctionnelle différente d'une même observation histologique à un nombre de jours différents post-partum peut être présente. (*Traduit par Dr Patrick Guay*)

INTRODUCTION

Grading systems for bovine endometrial biopsies have been used (1,2), however, well defined criteria, which are consistently correlated with subsequent reproductive performance, have not been well established in cattle.

In the previous paper (3) a summary of the histological assessment of endometrial biopsies, taken from dairy cows at day 26 and/or 40 postpartum was presented. Of 317 biopsies, at least subjective assessment was possible in approximately 95% of samples.

Including quantitative and subjective measures, as well as repeated measures from multiple areas within a biopsy, over 60 criteria were assessed from each biopsy. In this paper, the relationships among criteria are reported. Correlations between criteria within a biopsy, between the previously gravid and nongravid horns at day 26 and day 40, and between days within a horn were examined to identify: 1) those criteria which were consistently associated with other measurements, 2) criteria which were similar between horns and/or days within a cow and 3) criteria which were unrelated to other variables. This analysis will address two objectives. First, a description of the interrelationships among criteria which can be assessed from endometrial biopsies, and second, identification of a shortlist of histological parameters to be included in subsequent analysis of associations with bacteriological culture results, clinical findings and reproductive performance (4–6).

MATERIALS AND METHODS

Ninety-seven cows which calved in a commercial dairy herd between April and August 1984 were studied. These cows were included in a concurrent study (7,8) in which each cow was treated with either 500 μg of cloprostenol (Estrumate, Coopers Agropharm Inc., Ajax, Ontario) or saline on day 26 ± 3 postpartum followed by 500 μg cloprostenol or saline

Department of Population Medicine (Bonnett, Martin, Etherington, Buckrell) and Department of Pathology (Miller), Ontario Veterinary College, University of Guelph, Guelph, Ontario N1G 2W1.

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on day 40 ± 3 postpartum. Prior to treatment the internal reproductive tract was assessed by palpation per rectum, endometrial biopsies were taken for histological and bacteriological assessment, and a milk sample was acquired for progesterone determination. Reproductive and performance records were compiled and stored using an on-farm microcomputer and later transferred to the University of Guelph mainframe computer.

Details of the endometrial biopsy technique, histological assessment and descriptive statistics for criteria were reported in the previous paper (3). Briefly, subjective and quantitative criteria describing characteristics of the overall biopsy specimen, lumen, epithelium, stratum compactum (SC), stratum spongiosum (SS), glands and other tissues were analyzed. The horn which was larger on palpation per rectum was designated as the previously gravid horn.

ANALYSIS

Pearson product-moment correlation coefficients (r) were calculated to assess the relationship between findings, including both continuous (9) and binary and categorical variables (10). Results for each variable were compared with all other variables from the same biopsy, with all variables from biopsies from the opposite horn at the same day and from the same horn at the second day. Only coefficients which were significant at $p \leq 0.05$ are reported.

RESULTS

In total, 97 cows were biopsied. Of these 62 were biopsied on both day 26 and day 40, 23 cows were sampled only on day 26, and 12 were biopsied only on day 40. Therefore there were 85 cows biopsied at day 26 and 74 at day 40. One day 26 biopsy (nongravid horn) was lost in processing for a total of 317 biopsies assessed.

The subjective assessment of readability and the number of measurable areas of epithelium showed strong within biopsy correlation (Table I). There was some between horn correlation of readability assessments (biopsy quality) at day 26, but none at day 40. There was significant correlation between subjective assessment and epithelial counts at day 26 with both variables at day 40 in the gravid horn, but none within the nongravid horn.

There was no correlation of lumen inflammatory rating between horns at day 26, or within horn between days. There was significant correlation between horns at day 40 ($r = 0.4$).

TABLE I. Simple correlation coefficients (significant at $p \leq 0.05$) between readability assessments (subjective and epithelial counts) from endometrial biopsies

Within biopsy:		
	Epithelial counts	
Subjective[a]		
26G	0.80	
26N	0.57	
40G	0.80	
40N	0.67	
Between horns at day 26:		
	Nongravid	
	Subjective	Epithelial counts
Gravid		
Subjective		0.33
Epithelial counts		0.23
Between days within gravid horn:		
	Day 40	
	Subjective	Epithelial counts
Day 26		
Subjective	0.33	0.33
Epithelial counts	0.34	0.26

[a]26,40 = days postpartum; N = nongravid horn; G = gravid horn

TABLE II. Simple correlation coefficients (significant at $p \leq 0.05$) between epithelial height and cell counts from endometrial biopsies

Within biopsy:	Mononuclear cells	Segmented cells	
Height 26G[a]		0.44	
Height 26N		0.60	
Height 40G		0.58	
Height 40N		0.60	
Between horns:	Height 26N	Mononuclear 26N	Segmented 26N
Height 26G	0.47		0.41
Mononuclear 26G			
Segmented 26G	0.39		0.67
	Height 40N	Mononuclear 40N	Segmented 40N
Height 40G	0.44		0.48
Mononuclear 40G		0.52	
Segmented 40G	0.46		0.64
Between days:	Height 40G	Mononuclear 40G	Segmented 40G
Height 26G			0.50
Monunuclear 26G	−0.44		
Segmented 26G	0.42		0.51
	Height 40N	Mononuclear 40N	Segmented 40N
Height 26N	0.50		0.56
Mononuclear 26N			
Segmented 26N	0.43		0.61

[a]26,40 = days postpartum; N = nongravid horn; G = gravid horn

The simple correlation coefficients for epithelial quantitative variables within biopsy, between horns and between days are shown in Table II. Within biopsy there was a strong correlation of average height of the epithelium and number of segmented cells. There was moderate correlation of height between horns for both days.

The number of segmented cells was also correlated between horns. Height and number of segmented cells in the epithelium in one horn were correlated with both variables in the other horn.

For mononuclear cells in the epithelium, there was no within biopsy or between day within horn correlation and no association between horns at day 26. At day 40 there was strong correlation between the gravid and nongravid horn in the number of mononuclear cells (Table II).

The number of segmented cells in the epithelium at day 26 was correlated positively with height and number of segmented cells in the same horn at day 40. Height at day 26 was correlated with segmented cells at day 40, in both the gravid and nongravid horn. Height in the nongravid horn at day 26 was correlated with height in the same horn at day 40. In the gravid horn, the number of mononuclear cells at day 26 was negatively correlated with height at day 40 (Table II).

There was significant correlation of the subjective rating of epithelial inflammation between horns at day 26 ($r = 0.33$) and at day 40 ($r = 0.64$), as well as within horn between day ($r = 0.37$ gravid, $r = 0.45$ nongravid).

The subjective assessment of epithelial inflammation was positively correlated with the height, number of segmented and number of mononuclear cells in the epithelium within all horns (Table III).

There was no within biopsy correlation between the number of mononuclear and segmented cells in the SC.

There was no between horn correlation at day 26, but at day 40 there was moderate correlation of mononuclear cell counts ($r = 0.5$) and very strong correlation of segmented cell counts ($r = 0.9$) in the SC between the gravid and nongravid horn. The number of mononuclear cells was correlated within the gravid horn between days ($r = 0.41$), but there was no similar association with segmented cells, and no between day correlation of cell counts in the SC in the nongravid horn.

The subjective inflammatory rating for the SC was strongly correlated between horns within day ($r = 0.5$). There was weak correlation between days within horn. There was strong correlation of the subjective appraisal of inflammation in the SC with the number of mononuclear cells within biopsy, but correlation of inflammation with segmented cells was weak except in the nongravid horn at day 40 (Table IV).

Associations between lymphocytic foci in the SC and other variables within a biopsy are listed in Table V. Lymphocytic foci in the SC of the gravid horn at both days were correlated positively with foci in the SS of the same biopsy. Foci in the SC were generally positively correlated with the readability of the same biopsy. An increased number of lymphocytic foci in the SC of both horns at day 26 and the nongravid horn at day 40 was associated with an increased number of mononuclear cells in the epithelium. At day 26, SC lymphocytic foci were negatively correlated with the number of segmented cells in both the epithelium and the SC. However, at day 40 foci were positively correlated with epithelial segmented cells.

There was strong correlation of the numbers of lymphocytic foci in the SC between the gravid and nongravid horn at both days ($r = 0.60$ to 0.67).

Increased numbers of lymphocytic foci in the SC at day 26 in the gravid horn were associated with increased numbers of lymphocytic foci in the SC and SS of the same horn at day 40. Foci in the SC in both horns at day 26 were associated with an increased number of glands in the same horn at day 40.

The readability of the biopsy at day 26 was positively correlated with the number of foci in the SC at day 40, in the gravid horn.

Associations between lymphocytic foci in the SS and other variables within a biopsy are listed in Table VI.

TABLE III. Simple correlation coefficients (significant at $p \leq 0.05$) for epithelial measurements and the subjective assessment of inflammation in the same biopsy

Variable[a] Epithelial inflammation	Height	Mononuclear cells	Segmented cells
26G	0.47	0.37	0.45
26N	0.64	0.35	0.55
40G	0.42	0.51	0.63
40N	0.27	0.67	0.39

[a]26,40 = days postpartum; N = nongravid horn; G = gravid horn

TABLE IV. Simple correlation coefficients (significant at $p \leq 0.05$) for cell counts and the subjective assessment of inflammation in the stratum compactum within a biopsy

Variable[a] Stratum compactum inflammation	Mononuclear cells	Segmented cells
26G	0.53	0.25
26N	0.64	
40G	0.54	
40N	0.67	0.32

[a]26,40 = days postpartum; N = nongravid horn; G = gravid horn

TABLE V. Simple correlation coefficients (significant at $p \leq 0.05$) between stratum compactum lymphocytic foci and other variables, within biopsy

	Stratum compactum lymphocytic foci			
Variable[a]	26G	26N	40G	40N
Readability				
Subjective 26G	0.34	0.22		0.25
Counts 26G	0.29			0.30
Epithelial cell counts				
Mononuclear 26G	0.31	0.26		0.32
Segmented 26G	−0.26		0.32	
Epithelial inflammation				
26G				0.26
Stratum compactum cell count				
Segmented cells	−0.25			
Stratum spongiosum				
Lymphocytic foci	0.34		0.24	

[a]26,40 = days postpartum; N = nongravid horn; G = gravid horn

At day 26, within a biopsy, SS lymphocytic foci were generally correlated with subjective ratings of inflammation in the SS and in other parts of the biopsy. In biopsies from both horns at day 26 increased numbers of foci in the SS were associated with straight, as opposed to coiled, gland type. As well, increased numbers of SS lymphocytic foci were associated with increased layers of fibrosis around glands, in both horns at day 26.

At day 26, the number of foci in the SS of the nongravid horn was correlated with inflammatory ratings of the lumen, SC and SS in the gravid horn.

There were few correlations between the number of lymphocytic foci at day 40 and other variables from the same biopsy. However, increased numbers of foci in the SS of the gravid horn at both days were associated with increased numbers of foci in the SC of the same biopsy.

Lymphocytic foci in the SS of the gravid horn at day 26 were positively correlated with the number of foci in the SC and with the number of glands, and negatively correlated with inflammation in the SC and with fibrosis, in the same horn at day 40. Epithelial height and inflammation at day 26 were associated with increased numbers of foci in the SS at day 40 in the gravid horn.

In the nongravid horn, lymphocytic foci in the SS at day 26 were correlated with inflammation in the SS and around glands, and with smaller inner diameter of glands at day 40 in the same horn. In the nongravid horn, epithelial height at day 26 was associated with increased numbers of foci in the SS at day 40.

The subjective rating of inflammation in the SS was correlated between the gravid and nongravid horn at day 26 ($r = 0.39$) and at day 40 ($r = 0.52$). It was weakly correlated between days within the gravid horn ($r = 0.24$) and in the nongravid horn ($r = 0.35$).

The subjective assessment of the concentration of glands was correlated between horns within day ($r = 0.3$).

There was strong within biopsy correlation between the subjective measure and the quantitative assessment of gland concentration at day 26 in both the gravid and nongravid horns ($r = 0.61$ and $r = 0.48$). At day 40 there was moderate correlation in the gravid horn ($r = 0.39$), but not the nongravid horn.

TABLE VI. Simple correlation coefficients (significant at $p \leq 0.05$) between stratum compactum lymphocytic foci and other variables, within biopsy

	Stratum spongiosum lymphocytic foci			
Variable[a]	26G	26N	40G	40N
Readability (subj) 26G		0.26		
Epithelium				
Mononuclear cells 26G	0.28			
Height 26G		0.26		
Type				0.29
Inflammation				
Lumen	0.24			
Stratum compactum (SC)	0.24			
Stratum spongiosum	0.33	0.36	0.34	
Vessels		0.31		
Glands		0.25		
SC lymphocytic foci	0.34		0.25	
Gland type	−0.25	−0.36		
Layers of fibrosis				
Highest	0.30	0.32		
Lowest	0.38	0.25		
Subjective	0.23	0.23		

[a]26,40 = days postpartum; N = nongravid horn; G = gravid horn

The number of glands (both quantitative and subjective variables) was in general negatively correlated with layers of fibrosis. There was some positive correlation of the number of glands with the diameter of glands at day 26.

The number of glands was negatively correlated with gland inflammation. Both measures of gland concentration were negatively correlated with measures of inflammation in other parts of the biopsy (e.g. subjective assessment of lumen, SC and SS inflammation and cell counts).

At day 40, each variable for measurement of gland diameter in the nongravid horn was correlated with the same variable in the gravid horn ($r = 0.32$ to 0.46).

Subjective assessment of dilated glands was correlated within biopsy with gland inner and outer diameter ($r = 0.3$ to 0.5), which also were strongly correlated within biopsy ($r = 0.69$ to 0.83).

Subjective and quantitative variables for gland diameter were generally negatively correlated with gland inflammatory ratings.

There was significantly more dilated glands (subjective assessment) at day 40, and this correlated well with the quantitative measurement of gland diameter.

There was strong correlation between glandular inflammation in the gravid compared to the nongravid horn within day ($r = 0.60$ to 0.67). There was no correlation of gland inflammation between days within horn. The predominant cell type was mononuclear in all cases.

As mentioned above, glandular inflammation was negatively correlated with the number of glands. There was a strong correlation between glandular inflammation and the number of layers of fibrosis.

The quantitative measures of maximum and minimum number of layers of fibrosis around glands were correlated with the subjective assessment ($r = 0.3$). All three of these variables were positively correlated with most measures of inflammation in other parts of the biopsy.

There were no within or between day correlations for vessel inflammation.

Subjective assessments of inflammation from all parts of the biopsy (lumen, epithelium, SC, SS, glands and vessels) were in general highly correlated within biopsy and between horns within day. As well there was moderate to strong correlation for epithelial, SC and SS inflammation between days within horn.

DISCUSSION

The use of correlation coefficients was deemed to be an appropriate

analytic technique to use as an initial screen of the large number of histological findings, as a general measure of association between criteria was desired. However, when such a massive number of comparisons are performed, consideration must be given to the potential for spurious relationships to have significant correlation coefficients, simply due to chance. Thomas *et al* (11) discussed pitfalls inherent in such broadly applied "data-snooping", and outlined some approaches to preventing the possible over-reporting of spurious relationships. The approach taken in this study was to report all associations which were tested, acknowledge those significant at $p \leq 0.05$, but in general, to only accept as important those showing consistent relationships. It should be stressed that multiple comparisons have been employed as a data screening technique, and individual associations should be interpreted with caution.

It is perhaps worthwhile to consider the possible interpretations of a significant correlation between variables, or the lack thereof. Positive correlation between two variables, within a biopsy, might mean that the two criteria were in fact measuring the same thing (for example the subjective measure of concentration of glands and counts of gland cross sections). Positive correlation might indicate a direct cause and effect relationship. Alternately, it could be that the criteria measured two different characteristics which, however, respond in a similar manner to a third stimulus (a common cause). In the situation where results from opposite horns were correlated, any of the above three scenarios might have applied, and this could then be considered as support for the hypothesis that one biopsy from one horn is representative of the histological status of the other horn, at least for correlated variables. One possible common cause might be a hormonal stimulus, having a similar effect on a specific structure, regardless of horn. Positive correlation between criteria measured at different days could be related as above, or might indicate static characteristics of the uterus.

Variables which were not correlated within a biopsy would be assumed to be unrelated in any of the ways suggested above. A lack of correlation between criteria which were purportedly measuring similar structures in different horns, may indicate random distribution of that structure throughout the uterus, i.e. that there was as much or more variability within a biopsy or within a horn than between cows. It must be considered however, that this lack of correlation could reflect a true difference between the previously gravid and nongravid horns, and perhaps an expression of a different reaction to similar stimuli.

No association between similar criteria measured at day 26 and day 40 postpartum might be a reflection of within cow variability. Alternately, it could be an indication that the passage of time was long enough for a significant change to take place, resulting in no apparent relationship between the day 26 state and the situation at day 40 (6).

Lack of correlation of biopsy quality between horns at day 26, and correlation between day 26 and 40 for the gravid horn would indicate that there were differences between the gravid and nongravid horns which affected the ability to obtain good biopsies. This may be an indication that preservation of the epithelium and/or quality of the biopsy obtained from the postpartum bovine uterus is not a function of current physiological status, but is affected by previous state (gravid vs nongravid). It is hard to draw comparisons from the literature, as most studies have compared factors between the left and right horn, without specifying which horn had carried the previous pregnancy.

The lack of correlation of lumen inflammatory rating between horns at day 26 may indicate that there was an actual difference in presence of inflammatory exudate in opposite horns early postpartum. By day 40, the situation in the two horns had become more similar. As there was no significant difference in the number of biopsies with no epithelium at day 26 compared to day 40, loss of exudate due to processing would not account for the difference in association between horns at different days.

The strength and consistency of the relationship between epithelial height and segmented cell counts supports its acceptance as a true association. If the increased number of cells was due to a transverse section of tissue exposing a greater area to examination, a similar association of taller epithelium and an increased number of mononuclear inflammatory cells, as well as segmented cells, would be expected, and was not seen.

The association between epithelial height and segmented cells would not likely be a cause and effect relationship, but rather concomitant effects of a separate cause. Further analysis (4–6) showed that there was no correlation between height of the epithelium, or number of segmented cells in the epithelium, with either progesterone levels or clinical signs of estrus. Presumably, the height-segmented cell relationship was not associated with the stage of the cycle, and is therefore hypothesized to be related to an inflammatory process. This suggestion was supported by the positive correlation of epithelial height and number of segmented cells with the subjective rating of inflammation in the epithelium.

Based on the consistent correlations seen in this study, in general, inflammatory scores from one biopsy from one horn may be considered to be fairly representative of the situation in the opposite horn. Studer *et al* (1) reported this same relationship.

Cells counted in the epithelium could generally be distinguished as inflammatory cells. In the SC segmented cells could be specifically identified, whereas the designation of mononuclear cells included inflammatory and noninflammatory stromal cells. Especially in the SC this could account for variation of mononuclear cell counts within a biopsy or between concurrently taken biopsies. It would appear that the segmented cell counts may be a more specific measure and more consistent from sample to sample from a single cow.

The correlation of the subjective measure of readability and the number of quantifiable areas of epithelium at day 26 with the number of lymphocytic foci at day 40 in the gravid horn, and of these biopsy quality variables with SC foci within biopsy is perhaps difficult to explain, other than relating to there being enough tissue present to allow a count to be done. This hypothesis would not adequately explain the between day correlation. It may be

associated with characteristics of the previously gravid condition as for the readability variables.

It is possible that different significance should be attached to lymphocytic foci, depending on their location within the endometrium, based on the relationships seen in this study. Although there was correlation between foci and inflammation, the association was not strong enough or consistent enough, especially for foci in the SC to conclude that foci were only a reflection of infection status. Although lymphocytic foci have been suggested to be an indication of bacterial infection (1,2,12–14), lymphocytic nodules have frequently been found in cows from which no bacteria have been isolated (12,15). In addition, there seems to be a difference in relationships between foci and other variables at day 26 compared to day 40. It is possible that the functional significance of the number of lymphocytic foci is different depending on the time period under study. Analysis examining these different relationships using a multivariable approach is presented elsewhere (4,6). If there is a difference between day 26 and day 40, there could certainly be a difference between the postpartum, prebreeding and late breeding periods, and this could account for conflicting reports of occurrence and significance of lymphocytic foci in the literature.

Several variables could be consistently assessed and, for these, the results from one biopsy might be representative of the opposite horn. Epithelial height and segmented cell counts were highly correlated within biopsy and between horns and also associated between days. Subjective assessment of inflammation in the epithelium and/or SC would generally identify biopsies which had any inflammation present. A biopsy which had inflammation at day 26 was likely to show inflammatory changes at day 40. Quantitative and subjective assessments of the number of glands, the dilation of glands and fibrosis around glands were highly correlated. There was a positive association between the number of cross sections and the diameter of glands, and both of these criteria were negatively correlated with fibrosis and inflammatory changes.

As discussed, there may be different functional significance of the same histological finding at a different number of days postpartum. Before the histological criteria which have been shown to be at least consistently assessable can be accepted as important diagnostically in identifying problem cows, their relationship with clinical findings, both prior and subsequent to days 26 and 40 postpartum, and with reproductive performance must be examined.

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