

The Use of Endometrial Biopsy in the Infertile Mare

P. A. DOIG, J. D. McKNIGHT AND R. B. MILLER

Departments of Clinical Studies (Doig) and Pathology (Miller), Ontario Veterinary College, University of Guelph, Guelph, Ontario. N1G 2W1 and Armstrong Bros. Ltd., Inglewood, Ontario (McKnight)

SUMMARY

The results of a study on endometrial biopsies obtained from 700 infertile mares are reported. Infiltrative endometritis was present in 51% consisting of a combination of an acute and chronic cellular response in 6%, mild chronic infiltrations in 35% and moderate to severe chronic infiltrations in 10%.

Demonstrable endometrial fibrosis was found in 88% of the mares with the majority having mild (51%) or moderate (35%) changes. The age of the mares and the average number of years barren gradually increased with the severity of endometrial fibrosis, as did the combined incidence of fetal loss (early embryonic death and abortion) during the three year period preceding the biopsy.

A significant decrease in foaling percentage for the year following the biopsy was observed with increasing severity of endometrial fibrosis. Chi-square analysis projected a decrease in foaling rate of 22.8% for each category increase in severity.

The number of years barren and the farm management system used also had a significant effect on foaling probability. The foaling rates for mares that were bred artificially (82%) under strict veterinary supervision using an extender incorporating gentamicin were significantly ($P < 0.05$) higher than for mares bred in a conventional manner under average management conditions (48%).

It was concluded that endometrial biopsy is a valuable diagnostic and prognostic technique. When the degree of fibrosis is used to predict foaling probability, the number of years barren and the breeding method to be used should also be considered to arrive at a more accurate prediction.

RÉSUMÉ

L'utilisation de la biopsie de l'endomètre, chez la jument infertile

Cet article rapporte les résultats d'une étude qui portait sur 700 biopsies utérines d'autant de juments infertiles. On décela des lésions d'endométrite, chez 51% d'entre elles; dans 6% des cas, on se trouvait en présence d'une inflammation à la fois aiguë et chronique; dans 35% des cas, l'endométrite était chronique, mais bénigne, tandis que dans 10% des cas, les lésions d'endométrite chronique s'avèrent tantôt modérées et tantôt plus marquées.

Une fibrose endométriale affectait 88% de ces juments; elle s'avéra légère, chez 51%, et modérée, chez 35% d'entre elles. L'âge des juments et la moyenne du nombre d'années sans gestation augmentèrent graduellement avec la gravité de la fibrose de l'endomètre, tout comme l'incidence combinée de mortalité embryonnaire précoce et d'avortement, au cours des trois années qui précédèrent le prélèvement et l'examen de la biopsie.

Au cours de l'année ultérieure à la biopsie, on nota une diminution appréciable du pourcentage des parturitions, diminution qui se révéla proportionnelle à la gravité de la fibrose endométriale. Le test chi-carré révéla une diminution de 22.8% des parturitions, pour chacune des trois catégories d'endométrite chronique.

Le nombre d'années sans gestation et le genre de régie exercèrent aussi une influence appréciable sur la probabilité de parturition. Le taux de parturition, chez les juments expérimentales qu'on insémina, sous étroite surveillance vétérinaire, avec du sperme dont le diluant contenait de la gentamicine, atteignit 82% et s'avéra plus élevé ($p < 0.05$) que chez les juments qu'on avait fait saillir de façon naturelle, dans des conditions de régie qui se situaient dans la moyenne, où il n'atteignit que 48%.

Les résultats de cette étude permettent de conclure que la biopsie de l'endomètre représente une technique valable, tant pour le diagnostic que pour le pronostic. Lorsqu'on se base sur le degré de fibrose endométriale pour prédire la probabilité d'une parturition, il faudrait aussi tenir compte du nombre d'années sans gestation et du mode de saillie, pour en arriver à une prédiction plus exacte.

INTRODUCTION

Infertility in the mare is a common and important clinical entity. The cause of infertility whether infectious or noninfectious can be determined in many cases by conventional methods of combining an accurate history with the findings on rectal palpation, vaginoscopy and bacteriological cultures. However, in a significant percentage of mares no apparent reason for infertility can be found on routine clinical assessment. Endometrial biopsy has recently been shown to be of both diagnostic (2, 3, 5, 8, 10, 11, 12, 13) and prognostic (5, 7, 8, 9) value in assessing the fertility of this type of mare.

The purpose of this study was to determine the value of endometrial biopsy in the evaluation of infertile mares in Ontario.

MATERIALS AND METHODS

Endometrial biopsies were obtained over a three year period from 700 mares presented to the Theriogenology Clinic, Ontario Veterinary College, University of Guelph or breeding farms in southwestern Ontario. The majority of mares sampled were Standardbreds (73%) with the remainder being Thoroughbreds (22%) or one of the pleasure breeds (5%).

Endometrial Biopsy Technique

Endometrial biopsies were obtained by methods previously described following rectal and vaginoscopic examinations (4, 6, 8, 10). In the absence of

palpable uterine abnormalities the biopsies were taken from either the left or right uterine horn over what is clinically termed the implantation region (8). Samples were fixed in Bouin's solution for one to three hours and then transferred to 10% formalin for transport to the laboratory. Routine histopathological sectioning followed and all specimens were stained with hematoxylin—eosin.

Assessment of Biopsies

The methods of endometrial biopsy assessment were similar to those previously described (8, 10). An evaluation of the stage of the estrous cycle was made and any evidence of glandular change (atrophy or hypoplasia) recorded.

Cellular infiltrations were evaluated as to cell type, distribution and severity. Cell types were recorded as polymorphonuclear (acute infiltrative endometritis) or lymphocytes and plasma cells (chronic infiltrative endometritis). The distribution of cells was recorded as to their location in endometrium (lumen, stratum compactum, stratum spongiosum or within the glandular lumen). The severity of cellular infiltrations was subjectively graded as mild, moderate or severe. Mild infiltrations were defined as those associated with a few cells, usually located in the stratum compactum or occasionally as isolated foci in the stratum spongiosum. Moderate infiltrations were found diffusely in the stratum compactum and/or frequently in the periglandular area or within the gland lumen. Severe infiltrations were found diffusely throughout the endometrium and frequently induced pleomorphism of the epithelium (8).

Endometrial fibrosis was graded into four categories: A) absent, B) mild, C) moderate or D) severe. Category A mares were free of demonstrable endometrial fibrosis. Those in category B had mild scattered but frequent periglandular fibrosis with less than four fibrotic foci in an average of three 5.5 mm linear fields (8). Category C mares had moderate diffuse endometrial fibrosis characterized by prominent glandular nesting and four or more fibrotic foci per 5.5 mm. Category D mares had severe obliterating

endometrial fibrosis throughout the entire biopsy specimen with associated glandular atrophy and/or cystic glandular change.

Severity of endometrial fibrosis was the main criterion used for classification. The presence of moderate or severe cellular infiltrations were also reported and used to alert the clinician to the possible need for treatment. Abnormalities, other than fibrosis, used to drop a mare to a lower category were evidence of nonseasonal endometrial hypoplasia, atrophy, or the presence of dilated glands. Classifications were made on the basis of the endometrial findings alone and were not influenced by the past history of the mare.

Collection and Evaluation of Data

Reproductive histories for the three years immediately preceding the biopsy were collected. Parameters included were age of the mare, number of years barren and incidence of fetal loss (early embryonic death and/or abortion).

In 92 mares classified as clinically normal, the results of uterine bacterial cultures, obtained by the manual technique (1) using a guarded culture instrument were compared with the biopsy findings.

Foaling data for the year following the biopsy was collected on 403 of the mares and analyzed according to the previously assigned category using the chi—square test. The data was also used to compare two farm management systems. Comparisons were made between mares bred on one farm under constant veterinary supervision (Group I) and those bred on other farms under variable management systems (Group II). Group I mares were Standardbreds bred on a single brood

farm having five stallions at stud. Artificial insemination, incorporating gentamicin¹ in the extender, was used exclusively. One veterinarian performed the biopsy and any necessary treatment, and also supervised the breeding of the mares. Group II mares were bred on many farms with considerable variation in management practices. The group consisted of 92 Standardbreds, 89 Thoroughbreds and 20 of pleasure horse breeding. The majority of mares in the group (70%) were served naturally, in some cases without direct veterinary supervision. The veterinarian taking the biopsy from mares in this group was usually not involved in supervising subsequent breeding.

RESULTS

Bacterial Culture Findings

Bacteria were recovered from 28 (30.4%) of the 92 uterine cultures taken from clinically normal infertile mares. The most common isolates were beta hemolytic streptococci (10/28) and *Escherichia coli* (11/28). Endometrial biopsy findings indicated that only five (18%) of the culture positive mares had evidence microscopically of neutrophils in the uterine lumen or endometrium. Of 64 mares that were negative on culture, three (5%) had a demonstrable neutrophilic response. Mononuclear cell infiltrations (lymphocytes and plasma cells) were found in 75% of culture positive mares and 48% of those that were culture negative (Table I).

Endometrial Biopsy Findings

Demonstrable endometrial fibrosis was found in 88.3% of the mares with the majority having either mild (50.9%) or moderate (35.1%) changes.

TABLE I
RESULTS OF UTERINE BACTERIAL CULTURE AND ENDOMETRIAL BIOPSY
IN 92 CLINICALLY NORMAL MARES

Group	Number (%) Mares		
	Total	With positive biopsy findings for:	
		Neutrophils	Mononuclear Cells
Culture positive ^a	28(100)	5(18)	21(75)
Culture negative	64(100)	3(5)	31(48)
	92		

^aIsolates included: Ten beta hemolytic streptococci; 11 *E. coli*; two *Staphylococcus aureus*; three *Klebsiella* spp.; ten other bacteria (*Proteus*, *Pseudomonas* spp., *Corynebacterium* spp.)

¹Gentocin, Schering Canada Inc., Pointe Claire, Quebec.

TABLE II
ENDOMETRIAL BIOPSY FINDINGS ON CLINICALLY NORMAL BARREN MARES SHOWING THE ASSOCIATION BETWEEN INFILTRATIVE ENDOMETRITIS AND DEGREE OF ENDOMETRIAL FIBROSIS (N = 700)

Category	Degree of Endometrial Fibrosis	Percent with Concomitant Infiltrative Endometritis Acute and Chronic ^a	Chronic ^b	
			Mild	Mod/Severe
A	Absent	3.5	19.3	0.0
B	Mild	3.8	37.8	10.0
C	Moderate	9.3	38.0	14.0
D	Severe	25.0	25.0	37.5
All Mares		6.0%	35.0%	10.0%

^aCombination of neutrophils and mononuclear cells.

^bMononuclear cells only.

TABLE III
HISTORY OF MARES ACCORDING TO CATEGORY OF ENDOMETRIAL FIBROSIS

Category	Number	Age		Years Barren		Preceding Three Yr Incidence of		
		Mean	Range	Mean	Range	EED ^a	Abortion	Combined
A	82	6.9	(3-20)	1.2	(0-3)	13.3	8.4	21.7
B	356	8.9	(3-25)	1.4	(0-7)	22.0	14.6	36.6
C	246	12.1	(4-25)	1.7	(0-9)	20.6	17.8	38.4
D	16	14.3	(6-22)	2.9	(1-5)	30.7	30.7	61.4
Total	700							

^aEarly embryonic death.

Cellular infiltrations were observed in 51% of the endometrial biopsies. A combination of acute and chronic infiltrative endometritis was observed in 6%, mild chronic infiltrative endometritis in 35%, and moderate to severe chronic infiltrative endometritis in 10%. Cellular infiltrations were less common in category A mares than in those with moderate (Category C) to severe (Category D) fibrosis (Table II).

Although there was a wide range in the age of mares in each category, increasing age seemed to accompany an increase in the severity of the endometrial fibrosis. Similarly, the average number of years barren gradually increased with the severity of endometrial fibrosis as did the inci-

dence of fetal loss during the three year period preceding the biopsy (Table III).

Fertility Prognosis

The foaling data of mares in each category for the year following the biopsy is given in Table IV. A significant decrease in foaling percentage was observed with increasing severity of endometrial fibrosis. Chi-square analysis projected a decrease in foaling rate of 22.8% for each increase in category of fibrosis ($p < 0.05$).

TABLE IV
FOALING PERCENTAGES OF MARES FOR EACH CATEGORY OF ENDOMETRIAL FIBROSIS

Category	Number Of		Foaling %
	Mares Bred	Number Foaling	
A	57	47	82 ^a
B	209	155	74 ^a
C	129	59	46 ^a
D	8	0	0 ^a
Total	403	261	64

^aThe differences are significant at $p < 0.05$ (chi-square test).

Data combining the degree of fibrosis with the number of years barren is presented in Table V. Comparisons could only be made within categories B and C due to insufficient variation in the number of years barren in the other groups. The number of years barren had a significant effect on foaling percentage in mares with mild (Category B) and moderate (Category C) endometrial fibrosis ($p < 0.05$).

The influence of farm management practices on foaling rates for mares in the various categories is given in Table VI. Mares in group I bred artificially under strict veterinary supervision, using an antibiotic semen extender, had significantly higher foaling rates (82%) than mares in group II bred naturally under less stringent management systems (48%) ($p < 0.05$).

Degree of fibrosis and number of years barren did not influence foaling rates in group I until the mares were rated as moderately fibrosed and barren two years or more. The foaling percentage of mares in group II was strongly influenced not only by degree of fibrosis but also the number of years barren ($p < 0.05$). Foaling percentages dropped dramatically in group II at the category of mild fibrosis and barren two years or more.

DISCUSSION

The findings in this study support previous observations that endometrial biopsy is a valuable diagnostic and prognostic aid in the evaluation of the infertile mare (2, 5, 8, 10). The technique is safe, easy to perform, and can be utilized by the practicing veterinarian.

TABLE V
FOALING PERCENTAGES OF MARES CLASSIFIED ACCORDING TO CATEGORY OF FIBROSIS AND YEARS BARREN

Category	Years Barren	No. Bred	No. Foaling	Foaling %
A	1 ^a	57	47	82
B	1	151	124	82 ^c
	2 or more	58	31	53 ^c
C	1	68	42	62 ^d
	2 or more	61	17	28 ^d
D	2 ^b or more	8	0	0
		403	261	64

^aInsufficient numbers barren two years for comparison.

^bAll mares barren two years or more except one.

^c^dThe differences in foaling percentages with the same superscript are significant at $p < 0.05$ (chi-square test).

TABLE VI
FOALING PERCENTAGES OF MARES CLASSIFIED ACCORDING TO CATEGORY OF FIBROSIS,
YEARS BARREN AND FARM MANAGEMENT

Category	Years Barren	Group I ^a			Group II ^b		
		No. Bred	No. Foaled	Foaling %	No. Bred	No. Foaled	Foaling %
A	1	34	32	94	23	15	65
B	1	101	87	86	50	37	74 ^c
	2 or more	18	15	83	40	16	40 ^c
C	1	33	25	76 ^d	35	17	49 ^e
	2	16	6	37 ^d	45	11	24 ^e
D	2 or more	0	—	—	8	0	0
		202	165	82% ^f	201	96	48% ^f

^aAll are Standardbred mares bred artificially on a single farm to stallions of high fertility using an antibiotic semen extender. Same veterinarian taking biopsy, carrying out treatment if necessary and supervising breeding.

^bVariable breeds and farm management systems. Majority (70%) natural breeding with or without veterinary supervision. Veterinarian taking biopsy not usually involved in supervising the subsequent breeding.

^{c-f}Foaling percentages with the same superscript are different at $p \leq 0.05$ (chi-square test).

ian with a minimum of equipment.

The type and incidence of various pathological changes observed in this study are similar to those reported by Ricketts (10). Evidence of endometrial histopathology was observed in over 90% of the subfertile mares in both studies. Endometrial fibrosis was the most common abnormality, being present in over 75% of the mares. Acute infiltrative endometritis was a less common finding in this study presumably reflecting the fact that mares with clinical evidence of infection were not included. Chronic infiltrative endometritis, seen in approximately 45% of mares is similar to the incidence reported by Ricketts (11).

The findings, comparing the results of uterine bacterial culture and endometrial biopsy support previous conclusions that positive uterine cultures are often not associated with histopathologic evidence of endometritis (8, 14). Kenny reported only 75% agreement between culture results and biopsy findings, and suggested that mononuclear (macrophage) infiltrations were probably the result of response to foreign matter such as medication rather than actual infection (8). Witherspoon *et al* (14) found that of 34 cases of positive uterine bacterial cultures, in only nine was there simultaneous evidence of endometritis on biopsy. In the present study on 92 clinically normal barren mares, positive uterine bacterial cultures were

obtained on 28 (30.4%). Since histological evidence of an acute inflammatory response was observed in only 18% of the culture positive mares as well as 5% of those that were culture negative, there is reason to question the excessive emphasis often placed on results of single cultures from clinically normal mares. In the absence of biopsy results, the history of the mare, clinical findings, number and species of bacteria isolated and findings on cytology should all be considered when interpreting uterine cultures.

Classification of endometrial degeneration in this study was patterned after that proposed by Kenny (8, 9). Initially the types of endometrium found were classified into one of three categories as previously described but it was soon felt that four different categories could be recognized. The main difference between this rating system and the one proposed by Kenny is that an additional category (D) was added for mares with severe obliterating fibrosis. Moderate to severely fibrosed mares were divided into two categories rather than one, as proposed by Kenny. Since a modified classification method was used, categories were given an alphabetical designation to avoid any confusion with the numerical rating system (8, 9) which is currently in use throughout the United States.

The average age of mares placed in the various categories increased with

the degree of endometrial fibrosis, however, there was a wide range of age in each category. No fibrosis was apparent in one 20 year old mare while a six year old mare was found to have severe fibrosis.

The higher incidence of early embryonic or fetal death previously associated with moderate to severe endometrial fibrosis (8) was confirmed in this study. Mares with severe endometrial fibrosis had an incidence of combined fetal loss (early embryonic death or abortion) that was three times that recorded for mares without evidence of endometrial pathology.

The degree of endometrial fibrosis had a significant effect on foaling rates through each of the four categories similar to that reported previously using a three category system (8, 9). On the basis of these findings there appears to be justification in using a four category system.

The number of years barren also had a significant effect on foaling rates in those categories where comparisons could be made (Cat. B and C). The magnitude of difference in foaling rates between mares with the same degree of fibrosis was a surprising finding and suggests that factors other than fibrosis play an important role in infertility. The use of "number of years barren" with degree of fibrosis in the foaling prognostication is recommended for a more precise prediction of foaling probability.

Significant differences were also observed in foaling rates for mares bred under different farm management systems. The rates obtained in mares bred artificially under strict veterinary supervision with extended semen incorporating an antibiotic were not affected by the degree of endometrial fibrosis or years barren until moderately fibrosed and barren two years. These findings indicate that optimal breeding management can overcome the detrimental effects of mild endometrial fibrosis and even moderate fibrosis providing the mare is barren for only one year.

Conversely, under "average" management systems, primarily using natural breeding, the degree of fibrosis and years barren had a significant effect on fertility. Since one of the major differences between the two management systems was the degree of

hygiene practiced at breeding (artificial insemination using an antibiotic semen extender vs natural cover), the findings suggest that one of the factors other than fibrosis involved in infertility may be lowered resistance of fibrosed mares to bacterial exposure at the time of breeding.

It is apparent from the data that problem mares (barren two years with mild fibrosis or with moderate fibrosis regardless of years barren) should be bred under strict veterinary supervision, preferably using a "minimal contamination technique" (9) if acceptable foaling rates are to be achieved.

In conclusion, endometrial biopsy in the infertile mare is a valuable diagnostic and prognostic technique. When the degree of fibrosis is used to predict foaling probabilities, the number of years barren and the method of breeding should be taken into account to arrive at a more accurate prediction.

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