

# Effects of Incidence and Degree of Endometrial Scarring on Fertility in Dairy Cattle

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Infertility costs the dairy industry in the U.S. approximately \$1.266 billion or \$116.25 per cow each year. This loss is based on milk and calf losses, additional breeding services and semen, heifer replacement expense, medication and veterinary services.<sup>1</sup>

As bovine practitioners we use our fine-tuned palpating expertise almost entirely, and rightfully so, to evaluate an infertile problem along with visual examination of the vagina and its discharges. Then treatment is done accordingly by what is felt and seen.

Some practitioners will occasionally run bacterial cultures especially if the discharges indicate infection. This is usually done after several attempts of antibiotic therapy fail or when entering a problem herd for the first time.

These above procedures are necessary to properly evaluate routine infertile problems in a herd, but for the clinically "normal" repeat breeder, that is considered valuable by the client from a milk production and a genetic point of view, practitioners have to do more diagnostically. Bovine endometrial pathology should definitely be included diagnostically and prognostically, before days open become too extensive and culling too evident.

Before delving into endometrial pathology, namely endometrial scarring and endometritis, the endometrial biopsy technique should be discussed briefly. A more detailed article on biopsy technique appears in the May 1983 issue of VM/SAC. The uterine biopsy, first used in the bovine in 1948, is not a common diagnostic tool. This procedure is definitely for the researcher, but is also for the bovine practitioner that is interested in the valuable repeat breeder, the dairyman's economy and one's personal satisfaction of completing as thorough diagnosis as possible.

The uterine biopsy is a safe and easily performed technique.<sup>2 3</sup> Repeated biopsies have been shown to cause no adverse effects on the cow's reproductive capabilities.<sup>4</sup> Routine biopsy sampling in the dairy herd is actually not

practical and too expensive, but should always be considered and available when valuable, high-producing repeat breeders are to be evaluated.

## Technique

The restrained cow to be biopsied is scrubbed thoroughly with disinfectant soap and warm water in the perineal area to minimize the introduction of infection. Then the area is disinfected with a 70% isopropyl alcohol, followed by a tamed iodine solution. There is always a small risk of introducing an infection or of aggravating a previous infection especially when the biopsy is procured during the luteal phase,<sup>5</sup> which is the optimal time to take the biopsy in the bovine.

Several biopsy instruments are available for use. In our study<sup>3</sup> we have used exclusively the alligator type forceps similar to the instrument used in the mare but with a slightly smaller insertion end. This enables the operator to enter the uterus more easily during the luteal phase.

The instrument should be cleaned well and sterilized between uses, especially between farms. While taking samples, the instrument may be kept in a bucket of warm, Roccol-S solution.

Biopsy samples are routinely taken from three sites in the cow, the left and right horns and the body. Samples from one or two sites in each horn or body of the uterus are generally representative of that section.<sup>5 6 7</sup>

The instrument with the jaws closed is advanced through the vagina and cervix into the uterus and guided with the other hand by rectal manipulation. The instrument's jaws are opened. Tissue specimens are collected from each horn 3 to 6 cm. anterior to the bifurcation of the uterus on the medial surface or at a selected palpable abnormality. Body samples are obtained as close to the cervix as feasible. Cervical samples may also be taken if cervicitis is suspected according to the size of the cervix on palpation.

The operator presses the uterine wall into the open jaws with the hand in the rectum. It is wise to hold the finger in position while closing the jaws to prevent slipping of the endometrium.

Care is of utmost importance in removing the delicate

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tissue from the biopsy instrument. Artifacts may be easily produced that may hinder true histopathological evaluation. The tissue, removed with fine forceps or a needle point, is immediately immersed in a fixing solution, that is at least 10 times the volume of the sample, to prevent drying out. Additional artifacts may be produced if tissues are allowed to dry out. A 10% buffered formalin solution was used in the study.

The container used for collection should be liquid-tight, contain only one sample, and be well identified as to section and animal.

Tissue sample processing can be done at any commercial, state or private laboratory equipped to make histological tissue slides. For routine evaluation, the slides were stained with hematoxylin and eosin.

Pathologists familiar with bovine endometrial histology and histopathology will be able to evaluate the pathology present. With the interpretation of the findings at hand, the bovine practitioner will be able to give a more realistic diagnosis and effective prognosis for the valuable repeat breeder.

In our study biopsy, culture swabs and blood samples have been collected since July 1979 on all lactating cows in the University of Maryland's dairy herds at 30-37 days postpartum with each subsequent pregnancy. Rectal palpation findings were also recorded at the same time. Gross examination of the reproductive tracts were examined and recorded, and additional tissue samples were collected, at necropsy.

The study is continuing on the remaining 45-50 cows that were charter members in the project. First calf heifers, entering the herd 28 months after the study was started, were not included in the study.

The purpose of our study was to determine the incidence and degree of endometrial scarring in all lactating cows, both fertile and infertile, in a dairy herd. From a diagnostic point of view, the bovine practitioner needs this type of information in order to truly evaluate a report on a biopsy sample. As we progress we will see that clinically reproductive cows usually have some endometrial pathology present.

We are also interested in the subsequent relationship of endometrial scarring to fertility.

#### *When to Biopsy:*

A biopsy can be taken at any time. Although due to normal cyclic functional cellular variations during estrus,<sup>5</sup> it is recommended that the biopsy be taken approximately 5-10 days post estrus. If the biopsy is taken during estrus, acute endometritis, if present, can not be truly evaluated because the inflammatory reaction may be transient.

In order for us as practitioners to fully understand and communicate well with our clients as to the findings of an endometrial biopsy, we should refresh our basic knowledge of the normal endometrium.<sup>5</sup>

The uterine glands are located in the intermediate and

basal areas of the endometrium embedded in connective tissue trabeculae which transverse the loose endometrial stroma. These trabeculae consist of connective tissue fibers and fibroblasts plus small round cells (lymphocytes, plasma cells, mast cells and pigment-bearing macrophages). These free cells are seldom present, at least, in large aggregated numbers in the normal endometrium.<sup>5</sup>

Abnormalities of the endometrium are divided into two categories:

1. Gland cells proper
2. Connective tissue around the glands.

Cystic glands occur with wide branched lumina with low cuboidal or flattened epithelium. Usually with cystic glands the periglandular connective tissue was not altered.<sup>6</sup>

Cystic glands were found in 10-12% of cows.<sup>6 8 9</sup> In our study on Parity I dairy cows 8.3% showed cystic glands which, in turn, on subsequent pregnancies showed varying degrees of periglandular encapsulation.<sup>3</sup>

Lymphoid nodules, another uterine abnormality, are masses of small lymphocytes in the stroma that often have a fibrous connective tissue capsule around them. They do not appear to interfere with normal pregnancy, but do appear to be more prevalent in poor breeders than normal breeders. When present they are distributed throughout the entire uterus. They may be a sequel to puerperal infection.<sup>5</sup>

Endometrial scarring is the most frequent abnormality of the bovine endometrium. With most modification of the periglandular connective tissues, the gland cells proper differed very little from the normal gland cells. The lumen of the gland tend to increase in size. The connective tissue is hypertrophied and distinctly modified. The fibroblasts become spindle-shaped with slender nuclei and start forming a distinct circling or encapsulating formation around the glands. This encapsulation distinctly differentiates these glands from neighboring normal glands.<sup>6</sup>

Initially we were interested in determining the number of cows in a dairy herd that showed endometrial scarring or periglandular fibrosis. In a recent publication,<sup>3</sup> incidence and Degree of Endometrial Periglandular Fibrosis in Parity I Dairy Cows, the data of a 3 year study was tabulated. Of the 112 first-calf heifers biopsied at 30-37 days post partum, 3 heifers showed normal endometria; 3 had increased lumen size of the glands; 9 showed cystic glands with shallowed epithelium; while 97 of the 112 (97.3%) showed varying degrees of endometrial scarring in, at least, one or more sections of the uterus.

Eight out of 10 heifers biopsied, that had been inseminated once or twice but did not conceive, showed mild encapsulation of fibroblasts around the majority of the endometrial glands.<sup>10</sup> In another study 80% of poor breeding heifers that never conceived showed some type of abnormality.<sup>6</sup>

When including all cows in the study, 19% of the total cows showed normal endometria in all three sections, likewise, 81% displayed fibrosis or scarring in, at least, one

section of the uterus.

After finding that endometrial scarring appeared to be quite prevalent in both good and poor breeders in a dairy herd, we became concerned as to the degree of scarring and to what extent, if any, these variations had on conception.

Uterine glands were graded in part, according to the Studer and Morrow numerical rating system:<sup>11</sup>

1. No visible lesions
2. Increased lumen size
3. Some cystic, with shallow epithelium
4. Endometrial scarring (periglandular fibrosis)

The varying degrees of encapsulation found in the present study prompted the authors to categorize endometrial scarring into the following:<sup>10</sup>

- a. **mild:** fibroblasts with elongated nuclei from a loose circle around the glands in a distinct pattern.
- b. **moderate:** fibroblasts again form a distinct circle as in the mild category, but the fibroblasts are more closely packed together. This is recognized by the increased number of slender nuclei in the area.
- c. **severe:** fibroblastic changes tend to involve more of the surrounding stroma than either the mild or moderate categories and seem to incorporate more glands. The nuclei become less pigmented and show varying degrees of cellular degeneration. The glands show more increased lumen size with flattened epithelium.

Many sections show combinations of the above categories. In most sections there appears to be no clear demarcation between categories, so classification is based on prominence of a category. Therefore, the degree of endometrial scarring is based solely on the visibility and clarity of the fibroblasts.

Due to the variability in scarring around the uterine glands in the 3 samples taken during each lactation at 30-37 days postpartum in different animals and even in the same cow, uterine glands were grouped by sections rather than by cow.

Uterine glands and surrounding connective tissues were normal in 36% of the sections while 64% showed some degree of abnormality.

The frequency that uterine glands and surrounding tissues decreased or increased in the amount of abnormalities during subsequent pregnancies is as follows:

|                      |     |
|----------------------|-----|
| Decreased —          | 12% |
| No change —          | 14% |
| Normal Increased —   | 9%  |
| Abnormal Increased — | 6%  |

The majority of changes occurred in categories normal through mild to moderate (Table 1).

TABLE 1. Uterine Glands

|                      |       |        |       |
|----------------------|-------|--------|-------|
| Normal               | 36.0% | —36.0% |       |
| Increased Lumen Size | 1.6%  | 4.6%   |       |
| Cystic Glands        | 3.0%  |        | 85.6% |
| Mild Fibrosis        | 29.0% | 45.0%  |       |
| Mild to Moderate     | 16.0% |        |       |
| Moderate             | 8.0%  | — 8.0% |       |
| Moderate to Severe   | 2.0%  |        |       |
| Mild to Severe       | 2.0%  | 6.4%   |       |
| Severe               | 2.4%  |        |       |

Cows were grouped according to the number of sections that showed normal and some degree of scarring. A comparison was made in each cow as to equality or inequality between the abnormal sections in the following two tables.

TABLE 2. Comparison of Degree of Endometrial Scarring Between Left and Right Horns and Body with Scarring in all 3 Sections of Uterus. Total 151

| Degree of Scarring   | Frequency | Percentage |
|----------------------|-----------|------------|
| Right = Left         | 115       | 76         |
| Right ≠ Left         | 36        | 24         |
| Right = Left = Body  | 94        | 62         |
| Right ≠ Left or Body | 57        | 38         |

TABLE 3. Comparison of Degree of Endometrial Scarring Between Two Scarred Sections in Cows with One Normal Section. Total 99

| Degree of Scarring       | Frequency | Percentage |
|--------------------------|-----------|------------|
| $X_1^* = X_2^{**}$ and 1 | 60        | 61         |
| $X_1 \neq X_2$ and 1     | 39        | 39         |

\* Represents one scarred section

\*\* Represents the other scarred section present

Table 4 shows the frequency of the degrees of scarring where only one section was abnormal.

TABLE 4. Variations in the Degree of Endometrial Scarring in Cows with Two Normal Sections. Total 50

| Scarred Section | Frequency | Percentage |
|-----------------|-----------|------------|
| Mild            | 36        | 72         |
| Moderate        | 10        | 20         |
| Severe          | 2         | 4          |
| Mild-Severe     | 2         | 4          |

From the preceding tables, the importance is obvious that tissue samples should be taken from the left and right horns and body from each cow that is biopsied. One biopsy sample is not representative of the whole uterus in the bovine as it is in the mare.<sup>12</sup>

## Conclusions

Endometrial scarring is a common pathological lesion in the bovine endometrium appearing in approximately 81% of the adult cows in a dairy herd. The degree of scarring varied greatly in severity between animals and even sections in the same individual. Mild and mild to moderate scarring were the more prevalent categories present.

The etiology of endometrial scarring has not been established. The fact, that mild encapsulation of fibroblasts are evident in approximately 80% of heifers that were inseminated once or twice without conception, indicates that pregnancy does not appear to be the initiating factor. Speculative factors that contribute to scarring may be hormonal variances, imbalance of intracellular homeostasis of the uterine glands and/or fibroblasts, anaphylactic sensitivity of endometrial tissues due to alien proteins, or nutritional imbalances.

As to the significance of endometrial scarring in bovine fertility, there is an apparent relationship between scarring and breedings per conception as the degree of scarring increases, especially when severe scarring is present. Due to the low numbers in the study of cows showing severe scarring, a significant difference was not tabulated. Although there are patterns in the data that would substantiate a relationship with conceptin and endometrial scarring. One example is the group of 18 cows that have had 4 biopsies over the past 4 years, likewise, 4 pregnancies during this period. These 18 cows would have to be considered normal reproductively, at least, clinically. Each of these cows had, at least, one normal section with a mild or mild to moderate fibrosis in the abnormal sections.

Histologic examination of the bovine endometrium not only evaluates endometrial scarring, but also cellular infiltrations in the endometrial stroma (endometritis).

Even though cellular infiltrations (endometritis) was not discussed in this paper, it is worthy to note that the inflammatory rating was the only category showing a statistical difference between cows culled for infertility and those culled for other reasons from the dairy herds at the University of Maryland.

**Endometrial scarring may contribute to infertility, but certain other factors such as cellular infiltrations, hormonal variations, embryonic abnormalities, vascular damage in the caruncles and, maybe, collagen in the connective tissues as in the mare may play a more decisive role.**

Even though more conclusive research must be done on endometrial scarring, we feel that the study indicates that the endometrial biopsy in the bovine is definitely beneficial for diagnostic and prognostic evaluation of the repeat breeder. This procedure enables the practitioner the opportunity to either eliminate or include endometrial pathology as a potential cause of the infertile problem.

Based on our study concerning endometrial scarring, we recommend the following:

1. Cows with mild scarring have a good chance of

maintaining a pregnancy.

2. Cows with varying degrees of scarring between the two horns, breed in the horn with the less severe scarring. Therefore, breed the cow when the ovulating ovary coincides with the more healthy horn.

3. Cows with one normal and one scarred horn breed in the normal with ovulating ovary coinciding even though the other may be only mild.

4. Cows with any degree of severe scarring have a poor chance of maintaining a pregnancy.

Based on the study concerning cellular infiltrations (endometritis), although not discussed in this paper, we recommend the following when the biopsy is taken 5-10 days post estrus:

1. Cows with acute endometritis have a poor chance of maintaining a pregnancy.

2. Cows with mild, chronic endometritis have a good chance of maintaining a pregnancy.

3. Cows with chronic endometritis have a poor chance of maintaining a pregnancy.

4. Cows with chronic endometritis in one horn, breed in the other horn when ovulating ovary coincides.

5. Cows with combinations of endometrial scarring and endometritis other than mild have a poor prognosis.

Endometrial biopsies from repeat breeders sent to the Bovine Biopsy Service at the University of Maryland by practitioners are processed for interpretation and recommendations. Recommendations are based on history, pathology found, days open, age and the genetic value of the animal.

### Case History #1

Four year old Holstein cow, that calved twice, has been open for 12 months. Ureaplasma had been isolated in the herd about one year ago. This cow showed vaginitis but the uterus appeared normal on palpation. She has been bred over 10 times with heat cycles averaging 25-26 days. No abortions were evident and progesterone levels were normal in mid-cycle. The cow was superovulated and flushed twice but no eggs were found. Both ovaries responded with palpable corpus lutei. Phenol red test failed to show the dye in the urine on two different occasions.

Biopsy Report: Submitted September 1983

Left Horn – mild chronic endometritis

Right Horn – section inadequate for proper evaluation

Body – mild chronic endometritis

Diagnosis: Relatively normal with minimal, random monocytic infiltration.

Recommendations: History suggests possible obstruction of the oviducts.

1. Recipient for embryo transfer – this may enable the animal through enlargement of the reproductive tract and increased natural secretions to dislodge any possible obstructions in the oviduct for future natural conceptions.

2. Dry off, if still milking, and induce lactation – breed on the second estrus. Since the right horn sample was not adequate for evaluation, it would be recommended to hope for conception in the smaller horn (more healthy horns); waiting for follicular development and ovulation on the corresponding ovary.

**Note:** The practitioner, who sent the biopsy to the University reported that an *Angus* embryo had been placed and is currently being maintained by the surrogate mother.

#### Case History #2

First calf heifer calved 16 months ago. She was superovulated and flushed approximately 4 months after calving. No corpus lutei were palpated. A parovarian cyst, evident on the right fimbria after flushing, gradually regressed over several months. The cow was bred several times after the flushing with 21-28 day heat cycles. Mid-cycle progesterone levels were low-normal. In July 1983 she was superovulated again with 2-4 palpable corpus lutei on both ovaries, but no eggs were recovered. In August 1983 the henol red test was done with the dye being excreted in the urine.

**Biopsy Report:** Submitted September 1983. Taken 10 days postestrus.

- Left horn – minimal suppurative exudate in lumen of gland and uterus.
- moderate edema in interglandular areas
  - diffuse mild to moderate monocellular infiltration with several small lymphoid nodules.
  - mild congestion of small vessels

Right horn – similar to left horn

- Body – less edema of interglandular areas
- several vessel lumens full of neutrophils

**Note:** edema and neutrophils present in the endometrium is suggestive of early estrus.

**Diagnosis:** Subclinical subacute to chronic endometritis

**Recommendations:**

1. Culture, if positive, medicate parenterally according to drug sensitivity for a 5-day period because of the presence of neutrophils in the blood vessels in the body sample.

**Note:** To flush the uterus with 1000 ml of sterile saline USP (gravity flow) may be a suggested local treatment. Since the biopsy was taken 10 days post estrus, the pre-

sence of neutrophils is pathological and indicative of a long standing uterine infection.

2. If a lumen culture is negative, a tissue culture (biopsy) would be suggested.
3. After medication has been completed and another estrus cycle over, take another biopsy at 10 days post estrus from all 3 sections to ascertain the cellular infiltration status. If neutrophils are still present, consider more therapy or cull depending on the value of the animal.
4. Dry off, if still milking, and induce lactation—breed on the 2nd estrus or wait for corresponding ovulation with the smaller horn.

**Note:** Endometritis present at 16 months postpartum does not look promising for conception especially with neutrophils present in the luteal phase, which possibly indicates a long standing infection. Estrus cycles of 28 days could possibly be due to embryonic deaths and, less likely, delayed ovulations.

For the bovine practitioner an endometrial biopsy is definitely a valuable adjunct to his or her scope of diagnostic capabilities and prognostic evaluation of the repeat breeder.

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