Abstract

A method of pregnancy diagnosis in the bovine, using crystallization patterns of cervical mucus and contraction of the vagina in response to oxytocin, is described. Long, fern-like crystals form during estrus but not at other periods of the cycle. The vagina contracts in response to oxytocin, either at estrus or during pregnancy. By combining a crystallization pattern other than the very marked pattern at estrus and a positive vaginal contraction under the influence of oxytocin, pregnancy was diagnosed.

Eighty-five per cent of 33 cows was correctly diagnosed at 15 days post-breeding. Errors on three cows may be justified by their elongated cycles and possible embryonic mortality. All pregnant cows were diagnosed correctly. Ninety per cent of 21 cows was diagnosed correctly at 27 days post-breeding.

The most common method of pregnancy diagnosis in the bovine is by rectal palpation. This method has little accuracy until after the first month of pregnancy, and even then it depends considerably on experience and skill of the technician. Efforts to develop a simple and accurate method for early pregnancy diagnosis in cattle have not been successful.

In this study, trials were conducted to determine the value of combined cervical mucus (1-4, 8, and 11) and vaginal pressure techniques (9, 10, and 13) as means of pregnancy diagnosis during the first month of gestation.

Experimental Procedures

A group of nine Hereford cows, three pregnant and six nonpregnant, was used for the preliminary study of the cervical mucus smears.

Each cervical mucus sample was collected with a sterilized inseminating catheter and glass vaginal speculum. A one-way hand pump was used to evacuate the plastic tube and the mucus spread on the glass slide. Each slide was placed directly in an incubator at 50°C until mucus was completely dry. The microscopic pattern of crystallization for each mucus sample was studied directly after preparation of the smear.

Daily collections of mucus were made on each animal for a period of approximately three months. In addition, collections of mucus samples from three animals were made at 12-hr intervals from one to two days prior to estrus until two days after estrus. Collections of mucus samples from two cows were made at 2-hr intervals from the time of estrus until 4 hr after ovulation. Estrus was detected by exposing the animals twice daily to a teaser bull.

Mucus patterns were classified into four groups according to shape and extent of crystallization.

Very marked patterns. This group was characterized by heavy crystallization of the entire smear. The crystals were long ferns, Figure 1. Microscopically, the crystals appeared as long needles.

Marked patterns. This group was characterized by crystallization of most of the dried smear. They were distinguished as short, curved, feather-like structures.

Mixed patterns. This group was characterized by the presence of some fern-like crystals accompanied by areas lacking crystallization. This group had a wide range of appearances, from well-organized patches of crystallization scattered among the negative areas to areas of a very few scattered crystals, Figure 3.

Negative. This group was characterized by a complete absence of crystallization, Figure 4.

A group of seven Hereford cows (two pregnant and five nonpregnant) was used to study the vaginal response after injecting oxytocin. The apparatus for measuring changes in vaginal pressure and the technique used were those recommended by Tavenner and Green (13). Thirty USP units of oxytocin were injected intravenously into the jugular vein and vaginal pressure recorded with a manometer connected to an inflated 60-cc volume rubber balloon placed in the anterior portion of the vagina. Pressures were measured at 1-min intervals for 8 to 10 min, beginning within 1 min after injection.

Precaution was taken to avoid inducing nervousness in the animals. Trials in which the cows showed great restlessness during the time of injection were omitted from the records.
Diagnoses of early pregnancy by using both the cervical smear and vaginal pressure techniques were conducted on 33 Hereford cows. Each cow had been inseminated artificially during the last few hours of the estrous period. After insemination, cows were returned to pasture. They were observed twice a day for any signs of estrus over a three-month period. Each bred cow was tested on Day 15 following breeding. Cows which did not come back in estrus

Fig. 1–4. Changes in the cervical mucous pattern during the estrous cycle. 1. Very-marked pattern near estrus. 2. Marked pattern pro- and post-estrus. 3. Mixed pattern. 4. Negative or absence of pattern. Magnifications 100×.
CERVICAL MUCOUS PATTERN

VERY MARKED

MARKED

MIXED

NEGATIVE

DAYS OF ESTROUS CYCLE

FIG. 5. Typical changes in the cervical pattern throughout the estrous cycle.

were retested on Day 27 following breeding. The technique of testing each cow consisted of two parts: First the dried cervical mucous smear was checked; secondly, the vaginal response to the oxytocin injection was determined approximately half an hour after taking the mucous sample. At the end of the experiment the animals were checked for pregnancy by rectal palpation. Cows varied between 40 and 60 days of pregnancy at palpation.

Results

Crystallization patterns of the 558 mucous samples studied varied according to the stage of the estrous cycle in which they were collected. The second and third cycles of Cow 1 are typical cycles, Figure 5.

Very marked crystallization patterns appeared as early as three days before estrus (avg 1.6) and continued until as long as seven days after estrus (avg 3.0). Range of duration was from 0 to 3 before, to 2 to 7 after estrus, for a total range of two to seven days (avg 4.6).

The marked pattern appeared before and after the very-marked pattern. With relation to estrus, the marked pattern occurred as early as five days before (avg 3.4) and continued to eight days after (avg 4.7). Average duration for the proestrous pattern was 1.5 days and for the post-estrous pattern, 1.7 days. The marked pattern was absent in some of the cows. One of the cows showed a wide fluctuation of marked and very-marked patterns for eight days after estrus. Normally, during the luteal phase of each cycle, the pattern fluctuated between mixed and negative, with mixed predominating.

The animal which had silent heat periods showed normal patterns of crystallization for an animal in estrus.

During periods of post-estrous bleeding, it was very difficult to detect any patterns. The mucus was mixed with blood and no crystals appeared after drying.

In the trials where collections of mucus at 12-hr intervals as well as at 2-hr intervals was made, it was found that although there was a wide variation in the shapes of the crystals it was very difficult to make a definite identification of ovulation through cervical mucous study.

Crystallization patterns in the pregnant animals varied between mixed and negative patterns. Few instances of marked patterns were observed. During the two estrous periods of the pregnant animals, patterns were either mixed or negative.

A vaginal pressure over 4 mm Hg maintained for approximately 2 min was considered positive and was recorded at all except one of the estrous cycles, in which the cervical mucus showed a very-marked crystallization pattern in nonpregnant cows. One cow with vaginitis did not follow this pattern. Marked, mixed, and negative crystallization patterns of the mucus were accompanied in all cases by a negative vaginal pressure in nonpregnant cows.

The pregnant cows gave a positive vaginal pressure in all of the trials. Pregnancy was
Table 1 presents diagnoses from 11 cows which came in estrus within a period of 22 days after insemination. With the exception of Cows 10 and 11, all cows were correctly diagnosed on the 15th day after insemination.

Table 2 summarizes findings on an additional 11 animals later diagnosed as nonpregnant by rectal palpation, with the exception of Cows 20 and 22. The cows diagnosed as nonpregnant agreed with rectal palpation. Cows 20 and 22 were diagnosed pregnant on the 15th and 27th days after breeding. By rectal palpation they were found to be nonpregnant. These two animals came in estrus after 36 and 59 days, respectively. It is believed that embryonic mortality occurred and that the animals were pregnant at the time of testing, since very close observations were made for estrus and none was observed prior to the time recorded.

Table 3 summarizes the work conducted on 12 cows diagnosed as pregnant by rectal palpation. All of the animals were diagnosed correctly through use of both cervical mucous and vaginal pressure techniques during the first month of gestation. Cow 29 came in heat after 39 days of pregnancy, although she gave a positive result at the 15th and 27th days of pregnancy. By rectal palpation she was proven to be pregnant at the time of this estrous period.

Accuracy of diagnosis for 33 cows was 85% at 15 days. Three cows at 15 days post-breeding were probably bred, as indicated by cycles from 36 to 59 days in length. The other two cycled normally and should be considered as errors in diagnosis. Accuracy in the 11 pregnant cows was 100%.

Nineteen of 21 cows were diagnosed correctly at 27 days after breeding. The two diagnosed incorrectly in this group were the same as those in the 15-day diagnosis, which had long cycles.

Discussion

Considerable variation occurred in the length of the 17 estrous cycles among the nonpregnant cows.
TABLE 3

Findings in cows diagnosed pregnant by rectal palpation

Findings on the 15th day after insemination

<table>
<thead>
<tr>
<th>Cow no.</th>
<th>Mucous pattern</th>
<th>Vaginal pressure</th>
<th>Conclusion</th>
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<th>Conclusion</th>
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* Nervous, no injection.

...cows. Fourteen normal cycles were recorded, with a variable length from 17 to 23 days. Three estrous cycles were accompanied by silent estrus. Rectal palpation of these animals during that time revealed normal follicular development and ovulation. Three estrous periods were accompanied by post-estrous bleeding, which occurred one to four days after onset of estrus and persisted about 24 hr. One estrous period was preceded two days by bleeding and bleeding continued 12 hr.

Two of the pregnant animals showed signs of estrus. Both animals were approximately four months pregnant at the time. The cervix of each was closed tightly, with no excessive mucous discharge. The mucous appeared slightly less viscous than that of animals in normal pregnancy.

Evidently there is a direct connection between the salt content of cervical mucus and the hormone levels existing throughout the estrous cycle (12). Estrogen causes a rise in salt content, yielding a larger and more dense crystallization pattern than does progesterone. During the period of transition from the estrogenic phase to progestational phase, and vice versa, these changes are manifest.

Oxytocin has been shown to cause contraction of the reproductive system of the cow in estrus [Evans and Miller (7) and Cupps and Asdell (5)]. Also, Dukes (6) and Petrych (10) showed contraction of the reproductive tract during pregnancy under the influence of oxytocin.

This test of pregnancy involves oxytocin and crystallization principles by using one as a selective procedure and the other as an elimination procedure. Crystallization patterns select animals not in estrus and the vaginal contractions eliminate the nonpregnant animals.

This diagnosis procedure has application in research where the scientist wishes to know whether an animal is pregnant in the early stages, or can be refined to study hormone levels as they fluctuate in the cycle. The rancher or dairyman may use the diagnosis in small numbers of cattle, but without refinement and simplification it would not be practical under range conditions.

Acknowledgments

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References


