SOME ASPECTS OF OESTRUS IN CATTLE, WITH REFERENCE TO FERTILITY ON ARTIFICIAL INSEMINATION

2. CRYSTALLISATION PATTERNS IN CERVICO-VAGINAL MUCUS

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SUMMARY.

A study of crystallisation patterns in the cervical mucus of five cows is reported. The cows had calved 5–70 days before the trial period. Throughout the 46 days' experimental period 224 observations were made.

Evidence of strong oestrogenic activity was obtained in 32.5 per cent. of observations made within five days before or after an observed oestrus. Only 14.0 per cent. of observations were indicative of pronounced oestrogenic activity in samples taken within 5–10 days before or after an observed heat.

In samples which could not readily be related to an observed oestrus, some 47.8 per cent. of the patterns reflected pronounced oestrogenic influence.

The significance of these results in relation to the detection of oestrus in cattle and in the study of atypical reproductive states is discussed.

I. INTRODUCTION.

While oestrus is readily recognised in the dairy cow under normal conditions, its partial suppression under unfavourable conditions may render its detection by conventional methods difficult or impossible. Such conditions seem to obtain in certain of the beef cattle breeding areas of Queensland In addition, there is evidence that the syndrome referred to as "enzootic anoestrus" is widespread in some dairying regions of the State (McTackett 1956). It is important in such cases to determine if oestrus is completely suppressed and if ovulation occurs.

Furthermore, the efficiency of vasectomised "teaser" bulls, with or without raddling, in the detection of oestrus in beef cows is suspect. The importance of improving current methods of detecting oestrus is immediately apparent, particularly in respect of the application of artificial insemination to beef cattle.

Fluctuations in the characteristics of cervico-vaginal mucus have been utilized in physiological studies in women (Seguy and Simonnet 1933; Lamar, Shettles and Delfs 1940; Papanicolaou 1945; Pommerenke 1946; Abarbanel 1946) to indicate oestrogen activity, ovulation and early pregnancy (Roland 1952). Of particular interest have been the variations in the pattern of crystallisation of the mucus. Garm and Skjerven (1952) noted the incidence of fern-like patterns in the dried mucus is related to the level of systemic oestrogens. On the other hand, Bone (1954) considered that the incidence of fern-like patterns is inversely related to the level of circulating progesterone. It has been reported (Rydberg 1948) that the crystallisation is due to the presence of chloride ions in the mucus.

More recently, Alliston, Patterson and Ulberg (1958) made a study of the crystallisation patterns of cervical mucus in relation to oestrus in beef cattle. They noted that the fern-like patterns of crystallisation become evident about 3·5 days before oestrus.

In view of the favourable reports of the sensitivity of the crystallisation pattern of cervical mucus to fluctuations in the oestrogen-progesterone complex, the phenomenon was utilized in an attempt to detect fluctuations in systemic oestrogens in cows disposed to anoestrus.

II. MATERIALS AND METHODS.

In a study of oestrous behaviour of cows in the Nambour region of south-eastern Queensland (Fallon 1958), it was noted that the majority of cows exhibited oestrus within 55 days after calving. Six cows which had histories of longer intervals to first heat in previous seasons were selected for the trial. The animals had calved normally within 10 weeks before the experimental period.

The techniques used in collecting, treating and evaluating the mucus samples were similar to those described by Alliston, Patterson and Ulberg (1958). The sampling procedure is similar to the rectal fixation technique of artificial insemination and was performed by competent inseminator-technicians.

It became evident as the trial proceeded that the susceptibility of the genitalia to infection during periods of luteal dominance (Casida 1952; Rowson 1952) may present serious problems in this work. It was necessary to administer medication to three of the animals on the 5th day of the trial and to six on the 16th day. There was no evidence that the therapeutic measures, consisting of intra-uterine infusion of 1 g. streptomycin sulphate plus 50 ml. sodium sulphadimidine in each case, affected the mucus characteristics of the treated cows. On the 19th day of the trial one animal was deleted because of its susceptibility to infection.

The samples were collected at approximately 8.00 a.m. on each day during the 46 days' period from Aug. 12 to Sept. 26, 1958. Scoring of the

crystallisation patterns was performed independently by three observers on each day of sampling. The scores finally allotted were determined by discussion between observers.

A classification system similar to that of Alliston, Patterson and Ulberg (1958) was used. Scores were allotted on the following basis:—(1) no pattern; (2) patterns around bubbles only; (3) typical patterns covering less than half of the total smear area; (4) typical patterns covering more than half of the total smear area, but not complete; (5) typical patterns over complete area of smear, both short and long ferns being present; (6) typical patterns over complete area, only long ferns being present.

Oestrus records were based on observations by the co-operating farmer, who had many years' experience in dairy farming and an intimate knowledge of the behaviour characteristics of his herd.

Results are presented in relation to the five animals which were studied throughout the trial period.

III. RESULTS.

Particulars concerning calving and oestrus dates are set out in Table 1. It will be noted that one animal (E) exhibited oestrus five days before the trial commenced. Another (B) failed to display clinical symptoms of oestrus within at least 67 days after calving. It is unfortunate in some respects that anoestrus was not more marked in the experimental group. However, such difficulties are by no means uncommon in trials which must be based on predicted behaviour. The atypical oestrous cycles recorded for cow D are not unusual in their occurrence (Fallon 1958).

Table 1.

BREEDING HISTORIES OF TRIAL ANIMALS.

Cow.	Calving Date.	Dates of Oestrus*.
A	Aug. 7	Sept. 10, Sept. 28
B	Aug. 4	
C	July 30	Sept. 13, Oct. 4
D	July 26	Aug. 21, Aug. 31, Sept. 20, Oct. 2
E	June 3	Aug. 7, (Aug. 31–Sept. 1)†, Sept. 19, Oct. 1

* Oestrus data complete to 10–10–58.

† Oestrus was recorded on 2 succeeding days.

A total of 224 observations on cervical mucus was made. The distribution of the observations throughout the various ratings is presented in Table 2. It will be noted that scores of 5 and 6, which may be regarded as indicating a very strong tendency towards the display of fern-like patterns, were recorded in 78 (representing 34·8 per cent.) of the observations.

Table 2.

DISTRIBUTION OF CERVICAL MUCUS CRYSTALLISATION SCORES.

Score.	Animal.					Total.
	A.	B.	C.	D.	E.	
6	1	2	2	2	4	11
5	8	24	14	10	11	67
4	15	10	13	12	15	65
3	11	4	4	9	8	36
2	3	2	4	2	1	12
1	7	3	8	9	6	33
Total	45	45	45	44	45	224

When the data are considered more specifically in relation to the incidence of oestrus (see Table 3) it is noted that 26 out of 80 observations (32·5 per cent.) made within five days before or after an observed heat were classified as 5 or 6. Only 7 out of 50 observations (14 per cent.) made within 5–10 days before or after an observed heat were rated as 5 or 6. These results are in general agreement with those reported by other workers and indicate the importance of the follicular phase of the oestrous cycle in the manifestation of the phenomenon concerned.

Table 3.

INFLUENCE OF STAGE OF OESTRUS ON CERVICAL MUCUS RATING.

Score.	Days Before and After Oestrus.			
	± 0–5.	± 5–10.	± 10–.	
6	7	..	4	11
5	19	7	41	67
4	25	25	15	65
3	15	7	14	36
2	3	4	5	12
1	11	7	15	33
Total	80	50	94	224

However, observations made during phases apparently less influenced by follicular development are particularly interesting. Some 47·8 per cent. of observations (45 out of 94) made more than 10 days before or after an observed heat were recorded as 5 or 6. Indeed, in the case of cow B, which was noted above to have exhibited anoestrus for at least 67 days after calving, 57·8 per cent of mucus samples collected during the anoestrous phase reflected strong oestrogenic activity.

Attempts at the detection of cyclic tendencies in the observations which were not related to observed oestrous cycles were without avail, although, as indicated above, strong oestrogenic activity is suggested in the bulked data.

As noted previously, short oestrous cycles are by no means uncommon and satisfactory fertility may be expected after such cycles (Fallon 1958), indicating that ovulation occurs in most, if not all, cases. Thus, it has been of interest to consider mucus ratings in relation to such short cycles. The relevant data are presented in Table 4. A marked difference in mucus ratings is evident between typical and atypical oestrous cycles.

Table 4.

CERVICAL MUCUS RATINGS IN RELATION TO THE OCCURRENCE OF OESTRUS ASSOCIATED WITH ATYPICAL CYCLE LENGTHS.

Days Before Oestrus.	Average Mucus Score.		Days After Oestrus.	Average Mucus Score.	
	Typical Cycles (n = 7).	Atypical Cycles (n = 3).		Typical Cycles (n = 7).	Atypical Cycles (n = 3).
10 ..	3·3	4·3	0* ..	5·8	3·5
9 ..	3·4	5·0	1 ..	4·8	2·5
8 ..	3·4	4·7	2 ..	3·6	3·0
7 ..	3·5	3·7	3 ..	2·6	3·5
6 ..	2·6	4·3	4 ..	3·2	2·5
5 ..	2·7	4·7	5 ..	4·0	4·0
4 ..	4·0	2·5	6 ..	3·5	1·5
3 ..	4·6	2·5	7 ..	3·8	..
2 ..	4·3	1·0	8 ..	2·5	4·0
1 ..	4·8	2·0	9 ..	3·25	3·0
0* ..	5·8	3·5	10 ..	3·5	3·0

* Day of oestrus.

Indeed, in the case of cow D (see Fig. 1), were a base heat taken at Aug 21 and, in the method of Alliston, Patterson and Ulberg (1958), observations taken from the 16th day thereafter, a more typical rise in rating occurs

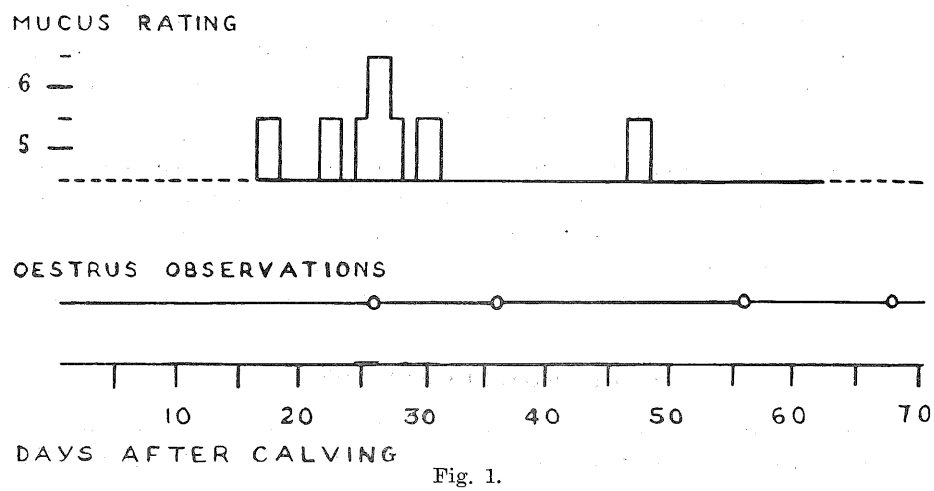

Fig. 1.

Cervical Mucus Crystallisation Patterns in Relation to Atypical Oestrous Cycles in Cow D.

at the 21st day, although no clinical symptoms of oestrus were noted. Furthermore, 9 days after the typical rise in mucus rating, to which reference has been made, a further heat with atypical mucus characteristics was recorded; it was followed in 12 days by an oestrus, for which no mucus ratings are available.

IV. DISCUSSION.

It is apparent that cervical mucus characteristics may be utilized as a valuable adjunct to current methods of detecting oestrus, particularly in relation to beef cattle breeding programmes. Certainly, if artificial insemination is being practised, sampling for cervical mucus beforehand would involve little additional time and labour for the competent operator. In the cow in which ovulation is imminent, the fern-like patterns in the cervical mucus are pronounced and readily observed.

There is ample evidence indicating that the changes in mucus characteristics during the follicular phase are essential for sperm penetration and viability in the mucus (Watson 1939; Campos da Pas 1951). However, a statistical relationship between mucus type and fertility remains to be established. There is ample evidence that tolerable levels of fertility may be obtained in the presence of cervical mucus markedly at variance in macroscopic appearance with "normal" oestrual mucus. This aspect is of further interest in relation to artificial insemination services, where inseminations are frequently performed 24 hours or more after the observation of oestrus and at a time when the peak mucus rating has passed.

The demonstration of high fern pattern ratings, indicating strong oestrogenic activity, during periods of anoestrus is of much interest. It serves, of course, to add further complication to the endocrinology of the syndrome. The complexity is accentuated when observations relating to short oestrous cycles are considered. It is apparent that the susceptibility of the cow's central nervous system to oestrogens (Asdell 1946) is variable and there is evidence that it may be completely suppressed under conditions of stress (Schaetz 1956; Fallon, unpublished data).

It is apparent that, in addition to their use in detecting specific changes in the reproductive state, crystallisation processes and other characteristics of cervical mucus (Blair and Glover 1956) may serve as useful aids in the elucidation of the more obscure aspects of bovine reproduction. The desirability of correlating the techniques more directly with hormone levels is obvious.

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