120. VARIATIONS IN CERTAIN PROPERTIES OF BOVINE CERVICAL MUCUS DURING THE OESTROUS CYCLE

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THE epithelium of the cervix and upper vagina of the cow and certain other animals produces a mucous secretion in which, according to Woodman & Hammond [1925], may be found a mucoprotein belonging to the class of mucin bodies containing mucoitin sulphuric acid, since they identified glucosamine in a specimen of cervical mucus. The chemical composition of this secretion does not appear to have been further investigated since this observation was made, but the scanty evidence available indicates that it may contain a mucopoly-saccharide-protein complex (see Meyer's [1938] classification of mucopoly-saccharides and glycoproteins).

Changes in the amount and physical properties of bovine cervical mucus were described by Woodman & Hammond [1925], who pointed out that at oestrus the secretion is plentiful, fairly fluid and capable of being drawn out into threads, whereas after oestrus it is considerably diminished in amount and less fluid. The amount of secretion, however, differs markedly in different animals and cannot be taken as a definite indication of oestrus. In pregnancy the secretion is very thick and adhesive, effectively sealing the gravid uterus, and Marshall & Hammond [1937] have described a rough, subjective test for pregnancy in the cow based on recognition of the characteristic stickiness of the cervical secretion. The work described in this paper forms part of a study of the chemical and rheological properties of bovine cervical mucus in different phases of the reproductive cycle, undertaken with a view to attempting to develop the mucin test of Marshall & Hammond [1937] into an objective test for early pregnancy in the cow.

As a first step it was considered necessary to study the changes in the properties of the secretion during the oestrous cycle in order to provide a standard with which to compare the behaviour of these properties during pregnancy. As has been shortly described in a preliminary note [Scott Blair *et al.* 1941], these studies have led to the development of a simple and rapid rheological test for oestrus or near-oestrus which may prove useful in the diagnosis of bovine oestrus in cases, and these are not infrequent [see Hammond, 1927], in which the external signs are not obvious even to a skilled herdsman, and also for predicting its imminence when, as in cold winter weather, it is to be expected that it may be easily missed on account of its short duration, particularly when it occurs at night.

Such studies might also be significant in connexion with the physiology of the passage of sperm into the uterus after coitus and might throw light on conditions which allow of bacterial invasion of the uterus through the cervix with consequent sterility.

The properties which have been included in this study are as follows:

- (1) Chemical properties (a) total nitrogen, (b) water content.
- (2) Rheological properties (a) viscosity, (b) flow-elasticity.

EXPERIMENTAL

Mucus samples

Samples were taken by the insertion of the hand into the vagina. The vulva was washed with a warm dilute solution of 'Dettol' and after one hand had been washed, disinfected and dried, liquid paraffin was applied to its back leaving the underside quite dry. Thus lubricated, the hand could be gently inserted into the vagina, extreme care being used and the line of least resistance always taken. It proved to be impracticable to obtain sufficient mucus for our purpose from the entrance to the cervix, so that the technique adopted as standard was to take as much mucus as possible from the vagina in the immediate vicinity of the cervix.

Throughout the work there was no indication that repeated sampling, twice a week as a rule and sometimes as often as three times weekly, led to any damage or infection of the reproductive tract likely to cause any change in the composition or properties of the cervical secretion or to hinder conception or disturb established pregnancy. For example, samples were taken from one of our cows at least once and usually twice a week for 10 weeks; she was then served, conceived to the first service and pregnancy proceeded normally despite the continued taking of mucus samples for a further 75 days, when she was slaughtered. It is inadvisable, and usually anatomically impossible, to take mucus samples by hand from the vaginae of nulliparous heifers.

Chemical analysis of secretions

Up to the present, chemical analyses have been confined to determinations of total N (micro-Kjeldahl) and total solids (by evaporation at 100°). Further studies are being made of the chemical properties of the secretions. Fig. 1 shows typical curves for individual cows which were sampled at regular intervals during one or two consecutive oestrous cycles and in one case well into pregnancy. The occurrence of oestrus as observed by the herdsman is indicated, and it will be seen that a sharp minimum in the total N content of the secretion occurs at about the time of oestrus, the N content falling to approximately one-tenth of the mid-cycle value. One cow, Clara 9, was served after samples had been taken over 2 cycles. Conception took place, as was known for certain when she was slaughtered 75 days later, and the periodicity in the total N content of the secretion immediately ceased, the values remaining at the relatively high level characteristic of mid-cycle samples. These observations indicate that a determination of the total N content of the secretion might be made the basis of a practical method for diagnosing oestrus, but, as will be seen, a far more rapid and simple test based on flow-elasticity was successfully devised so that these observations were not further extended.

Values for the total solids content of the samples from the same cows show a similar periodicity. As was to be expected from naked-eye observations of its physical properties, the water content of the secretion is highest at or near oestrus. This raises the question as to whether or not the fall in total N at oestrus is due merely to increase in water content. Calculated values (Fig. 1) for the total N content of the solid matter of the secretion indicate that this is not so.

The N content of the dry matter is minimal at oestrus with a value of 1–2%, while in mid-cycle it rises to a value of 6–10%. It may therefore be concluded that after oestrus nitrogenous substances enter the secretion. These are probably proteins arising from the breakdown of cellular debris from the epithelium of the reproductive tract, leucocytes, etc.

Fig. 1. Variations in total N of bovine cervical mucus during the oestrous cycle. Vertical broken line indicates observed oestrus and arrow denotes service. Dotted curves give percentage total N in dry matter (calculated).

Physical properties of secretions

(a) Viscosity

These secretions are by no means true fluids, that is to say, the viscosity is not independent of variations in stress and strain conditions. If a true fluid is caused to flow through a narrow tube, the amount of flow per second is proportional to the applied pressure. With the secretions, however, flow is relatively faster for high pressures than for low, and also the act of streaming itself causes a marked slowing down of flow for any given pressure (work-hardening). If it can be arranged, however, that the effective pressure on the flowing secretion increases at such a rate as approximately to offset the rise in viscosity due to the ever-increasing strain (the streaming), a viscosity can be obtained which, although by no means a constant of the material, should be sufficiently reproducible to be of value, especially since the viscosities of the secretions vary over a very wide range (sometimes of the order of 100,000 : 1) during the oestrous cycle.

Some years ago, a somewhat similar problem with honey led to the designing of a special viscosimeter [Scott Blair, 1937] in which a narrow glass tube is filled with the material to be investigated and is then emptied by means of a constant air pressure (P)¹ applied at one end. If the tube has a radius R and length L , and if the length of the column of secretion (which varies from L to 0 during the

¹ P must be quoted in dynes/cm.²

emptying) is described as l for any given time t , it is easy to show that for a true fluid, curves plotting $Pt/(L^2 - l^2)$ are linear and pass through the origin, and that their slope has only to be multiplied by $R^2/4$ to give the viscosity (η) in absolute units (poises).¹

Since the applied pressure at the end of the tube is kept constant, the stress which is actually shearing the column of secretion along the wall of the tube must be increasing rapidly (the area of effective wall being $2\pi Rl$). The secretions do not flow in the streamline manner of a true fluid, but, as is shown in Fig. 2,

Fig. 2. Flow-curves for oestrous and mid-cycle samples of bovine cervical secretions in Scott Blair viscosimeter.

the curves are remarkably linear, hence the fall in viscosity produced by this rising stress (which can be independently shown by comparing viscosities taken at two different air-pressures) must be very closely offset by the work-hardening effect. The secretion toughens as it is stretched along the tube. Oestrous samples show some tendency to downward curvature, since in them work-hardening is enormous; mid-cycle samples tend rather the other way, but there is rarely any difficulty in drawing a fair straight line through the points. Sometimes a slight zero error introduces a small intercept on the Pt axis, but the curve remains very nearly linear.

Viscosities determined in this way on secretions roughly homogenized by squeezing between two glass plates vary from about a poise in oestrus to about 1000 poises in mid-cycle, though much wider variations than this often occur. Remembering that the viscosity of water at room temperature is about 0.01 poises (1 centipoise) the range of viscosities is clearly visualized, though in view of the large numbers involved we prefer to plot $\log_{10} \eta$ in studying variations

¹ Small corrections in this formula are discussed in the original paper but may be neglected in the present work.

during the oestrous cycle. Fortunately the temperature coefficient of η is remarkably small and no temperature control is required.

Fig. 3 shows typical curves for individual cows during several cycles, and in one case well into pregnancy; the dotted lines indicate oestrus as observed by the herdsman (often with warning from the laboratory).

In obtaining these curves, it was not always possible to use the same pressure for all the samples on account of the wide variations in viscosity. Whenever possible, measurements were made at a pressure of 40 cm. of water; when this was not feasible, measurements were made at two different pressures, the viscosities at the standard pressure of 40 cm. of water being determined by

Fig. 3. Variations in viscosity of bovine cervical mucus during the oestrous cycle. Vertical broken line indicates observed oestrus and arrow denotes service. Points marked with a circle are somewhat unreliable due to errors in extrapolation at high viscosities.

extrapolation on $\log \eta / \log P$ diagrams, which are fortunately fairly linear. There is a tendency for these $\log \eta / \log P$ curves to be steeper in the case of oestrous samples. Viscosities determined in this way give a very fair indication of when a cow is in season or within a few hours either way of being in season, but the method is not advocated for practical purposes for the following reasons:

(1) The apparatus is too delicate and complicated for cowshed use and the test takes some time (at least 20 min. with calculation). It requires a certain amount of manipulative skill.

(2) On occasions, the secretions are apt to show misleadingly low viscosities when the cow is not in season. This is usually due to contamination of the mucus with urine because of urination before or during sampling, but occasionally it must be assumed to be due to other causes, such as catarrh associated with infection of the vagina. Samples contaminated with urine are generally detectable by appearance and smell.

(3) A much simpler and more reliable test is available, based on the measurement of 'flow-elasticity'.

(b) *Flow-elasticity*

If, during the extrusion of the column of secretion in the viscosimeter the pressure is suddenly released, the column recoils and flows back along the tube. For samples taken from cows in mid-cycle this recoil amounts to anything from 0 to about 4 mm. In animals actually in season, recoils are usually between 20 and 30 mm.; accuracy is low when recoil is much over 30 mm. This type of elastic recoil has been observed in other materials and discussed by earlier workers and was named by Szegvari 'Fließelastizität', which is generally translated 'flow-elasticity' [Szegvari, 1924; Ostwald *et al.* 1933].

Table 1. *Effect of various factors on flow-elasticity (ρ)*(a) Effect of varying the radius of the capillary on ρ

Tube no.	1	2	3	4
ρ (mm.)	18	18	17	18

The radii of the capillaries ranged from 1. to 2.5 mm. approx., No. 1 being the smallest. Larger capillaries are unsuitable for the test.

(b) Effect of rate of extrusion on ρ

Rate (cm./sec.)	0.5	0.7	1.7	2.0	2.7
ρ (mm.)	26	23	21.5	22	24

(c) Effect of initial length (λ_1) of column of mucus and length of column expelled before release of pressure (λ_2) on ρ

λ_1 (cm.)	13.0	9.5	9.5	9.5	6.0
λ_2 (cm.)	3.0	3.0	2.0	5.0	3.0
ρ (mm.)	12	20	20	21	28

In each of the above experiments the mucus sample used was taken from a cow at about the time of oestrus.

Flow-elasticity can be measured in a much simpler instrument than the Scott Blair viscosimeter. Experiments with secretions have shown (see Table 1) that the radius of the capillary, the pressure and the amount of extrusion before release can be varied over wide limits without effecting serious changes in the amount of recoil. The initial length of the column of secretion, on the other hand, is of some importance (see Table 1) and should be kept as constant as possible, though even differences of 100% in this are unlikely to cause confusion between oestrous and non-oestrous samples. Bearing these points in mind, a simple instrument which can be used in the cowshed for the prediction and diagnosis of oestrus has been designed and is depicted in Fig. 4.

Fig. 4. The Oestroscope.

A glass capillary tube (A) about 11 cm. in length and of internal diameter approximately 2 mm., is cemented on to the nozzle of an ordinary 2 ml. hypodermic syringe. There is a mark 10 cm. from the open end of the tube and between this mark and the cement join, a small side-tube (B) is sealed in at right-angles. The tube is filled up to the 10 cm. mark by suction, keeping the side-tube closed with the finger. The instrument, which we have called the 'oestroscope', is then

clipped in a horizontal position with the 10 cm. tube covering a mm. scale and the secretion is slowly expressed (rate of about 0.5–1.0 cm./sec.) until the end of the column reaches the 7 cm. mark. The apparatus is fastened to a board of sufficient thickness to ensure that the extruded secretion is able to form a kind of blob round the open end of the tube without touching the bench. As the meniscus reaches the 7 cm. mark, the finger is removed from the end of the side-tube and the pressure immediately falls to zero. The amount of recoil, which is practically complete after a few seconds, is recorded. The experiment may be repeated without refilling, but subsequent readings will not be as accurate as the first, and if the string of extruded secretion has touched the bench or even if it is hanging from the end of the tube, recoil may be seriously inhibited. Some secretions tend to form 'salients', i.e. the meniscus is not sharply defined. This can generally be rectified by varying the rate of extrusion. Dilution of the secretions with substantial amounts of water or urine may increase the recoil (designated as ρ in mm.), but in practice the effect is seldom very marked (see Table 2). It is safer however to discard samples known to be badly contaminated.

Table 2. *Effect of diluting mucus with water on ρ*

% mucus in mixture	Sample A ρ (mm.)	Sample B ρ (mm.)	Sample C ρ (mm.)	Sample D ρ (mm.)
100	2	4	6	2
40	2	7.5	7	3
18	1	10	20	7

As with viscosity, temperature effects are slight. The whole test takes only about 1 min. to complete. The oestroscope may be washed by rinsing in warm water; drying is not important, since the secretion does not mix quickly with traces of water. For field work, the glass capillary, on which a mm. scale is etched, can be cemented into a metal shank so constructed as to fit the nozzle of any 'Record' syringe in the same way as an ordinary hypodermic needle. This makes the apparatus more portable and should considerably reduce its cost.

Recoil values determined in samples from 4 cows at regular intervals over a period covering a number of oestrous cycles are shown plotted in Fig. 5. It is clear that the flow-elasticity of the cervical mucus varies regularly during the oestrous cycle, rising to a sharply defined maximum at, or very near, oestrus. One of the cows, Clara 9, was served after ρ -values had been studied over three complete cycles; the periodicity in ρ immediately disappeared and, as in the case of the other properties studied, the values remained at the level characteristic of mid-cycle. Seventy-five days after service the cow was slaughtered and pregnancy confirmed by recovery of the foetus.

The sharply defined periodicity in the flow-elasticity of the cervical mucus, correlated with the oestrous cycle, appeared to afford a basis for an objective method of diagnosis of oestrus, particularly since this property can be measured so rapidly and easily.

In order, therefore, to investigate statistically the value of flow-elasticity measurements as a practical method for the diagnosis of bovine oestrus, numerous determinations have been made on samples from cows in various reproductive phases. These results, which are summarized in Table 4, were obtained on samples brought to the laboratory and tested within a few hours, at most, of sampling. The apparatus in its simplest form is so designed that the measurements can be done in the cowshed immediately after the sample has been taken, though if desired samples can be brought to the laboratory and left for some

hours before testing. The results in Table 3 show that the ρ -values of samples kept at room temperature overnight seldom alter appreciably.

Fig. 5. Variations in flow-elasticity (ρ) of bovine cervical mucus during the oestrous cycle. Vertical broken line indicates observed oestrus and arrows denote service. Points marked with a circle are subject to a certain error due to inadequacy of sample.

Table 3. *Effect of keeping secretions for various times at room temperature on ρ*

Approx. time of keeping hr.	Sample A ρ (mm.)	Sample B ρ (mm.)	Sample C ρ (mm.)	Sample D ρ (mm.)	Sample E ρ (mm.)
1	5.5	5.0	4.0	6.0	2.0
18	5.5	—	3.5	5.5	2.0
42	5.5	4.5	4.0	7.0	—
66	—	—	4.0	15.0	—

Table 4. *Values of ρ in different phases of the reproductive cycle*

Group	...	I	II	III	IV	I + II
Highest ρ (mm.)		27.0	20.0	7.5	—	27.0
Lowest ρ (mm.)		5.5	6.0	0	1.0	5.5
Mean ρ (mm.)		14.7	10.9	2.4	2.5	14.0
Standard deviation		± 9.6	± 3.8	± 1.4	± 2.4	± 5.8
No. of samples		41	9	143	53	50

Table 4 summarizes the results of tests on 246 samples. Group I includes samples from cows observed by the herdsman to be in oestrus within a period of 24 hr. before sampling and 60 hr. after. These arbitrary time limits are taken as

corresponding, as far as is now known, to the average duration of a high ρ . Group II consists of samples from cows in which oestrus was not actually observed by the herdsman within the prescribed time limits, but its occurrence within these limits could be inferred from previously (and in some cases also subsequently) observed oestrous periods; group III contains samples from cows which were not in, or near, oestrus as defined by the above time limits at the time of the test and which were not pregnant; group IV consists of samples from cows believed by the herdsman to be pregnant. Some, but not all, of these have been subsequently verified by recovery of the foetus.

Comparing the figures for group I and groups I and II combined, with those for groups III and IV, it is clear that ρ for samples taken at or near oestrus is significantly higher than for samples taken in mid-cycle or pregnancy. On the basis of our data, therefore, it may be concluded that a ρ -value of 5 and upwards means that when the sample was taken, the cow concerned was either actually in season or within a few hours of its onset or its end. Should the ρ -value indicate oestrus and, on the trial being made, the cow refuse to accept the bull, a second test some hours later should indicate whether or not the first test was made shortly before or soon after oestrus. The form of the curves in Fig. 5 indicates that if the first test preceded oestrus the ρ -value some hours later will have risen, and if it followed oestrus the second ρ -value will be lower.

The high standard deviation for group I is due to the facts that samples were not always taken when the cow was exactly in oestrus but might be collected at any time during the arbitrarily chosen period around oestrus, and also that the maximum ρ is doubtless not the same for all cows nor in every oestrus for any given cow.

DISCUSSION

The criterion which we have used to define 'oestrous' samples is arbitrary and requires some explanation. The only correct criterion for a study of this kind is the test of whether or not the cow will accept the bull. For practical reasons it is not possible to apply this test nearly as frequently as would have been needed to get a statistically adequate body of data. In order to present an analysis of our data we were therefore obliged, in selecting our group of 'oestrous' samples with which to compare the 'non-oestrous' groups, to choose the arbitrary time-limits to which we have already referred.

Throughout this study, with the exception of a few cases in which the cow was served immediately after taking the sample, we were dependent on the observations of skilled herdsman who are able to recognize oestrus from the behaviour pattern generally shown by cows in season. It must be remembered, however, that oestrus as recognized by the herdsman by no means always corresponds exactly with the bull-acceptance criterion. The allocation of borderline cases between the groups, involved an element of subjectivity; each such case was therefore considered on its merits.

Preliminary inspection of the data gave the impression that ρ begins to rise above the normal mid-cycle level (which differs somewhat for different animals) about 24 hr. before oestrus and does not return to normal until about 60 hr. after it. These figures undoubtedly differ for different animals, but after considering all the evidence they seemed to give the best limits for our statistical analysis. The fact that ρ remains high for a longer period after than before oestrus may be due to two factors: (a) ovulation probably takes place some time after oestrus (Brewster & Cole [1941] give approximately $13\frac{1}{2}$ hr. after the end of heat and Hammond [1927] about 40 hr. after its start) and (b) mucus produced

at oestrus probably remains in the reproductive tract for some time after its secretion. Nothing is yet known of the effects (if any) of ovulation on the value of ρ , though it seems likely that there are such effects. It is clearly desirable to investigate this point and also to determine the way in which ρ varies hour by hour during the period of oestrus and for some time before and after. Facilities for such an exacting experiment have unfortunately not yet been available, though it is hoped that such difficulties may be overcome in the near future. In the meantime we believe that the treatment of our results on the basis just discussed does allow of the definite conclusion that ρ is highest at about the time when the cow will accept the bull and that therefore the oestroscope described provides a simple and rapid means of diagnosing oestrus.

Hitherto, there has been no adequate objective method of recognizing oestrus except putting the cow to the bull. According to Cole [1930], studies of vaginal smears afford no certain means of judging 'heat'. The presence of mucus in the smear, although associated with oestrus, is an unreliable criterion, a conclusion confirmed by Ferring [1937]. Quite frequently, especially in winter, cows show little or no external signs of oestrus even though ovulation takes place. There are several examples seen in Fig. 5 in which ρ showed a sharp maximum at a time at which, from oestrus dates and the known regularity of the oestrous cycle of the cow in question, it may be inferred that oestrus did occur despite the fact that the herdsman, although duly warned, failed to observe it. Unfortunately in all cases save two it was not possible to settle the matter definitely by serving the cows on these occasions. In one case, however, the cow stood to the bull while a semen sample was collected in an artificial vagina and in another instance the cow was served and became pregnant.

It is hoped that the oestroscope may prove particularly useful in such cases as those in which 'heat' would otherwise be missed and much valuable time wasted in getting cows into calf. Our results indicate that total N determinations would provide as reliable an indication of oestrus as the oestroscope but the latter affords a much simpler and quicker method.

Viscosity determinations, intermediate in complexity, are not so reliable, probably mainly owing to the serious effects on the viscosity of contamination with relatively small amounts of urine, such as would not cause a wrong diagnosis in the case of the other two methods.

SUMMARY

1. Variations in total N, dry matter, viscosity and flow-elasticity of bovine cervical mucus have been studied during the oestrous cycle.
2. Total N, dry matter and viscosity reach minimum values at about the time of oestrus; flow-elasticity shows a marked maximum.
3. Variations in total N in the secretions are great enough to serve as the basis of a test for the diagnosis of oestrus. The drop in percentage of total solids at oestrus is quite insufficient to account for the differences in total N observed. It seems that soon after oestrus nitrogenous substances enter the secretion.
4. The secretions are not true fluids but a viscosity value can be determined under arbitrarily standardized conditions, and this value could serve as the basis for a test for the diagnosis of oestrus were it not for the fact that various factors, especially comparatively slight contamination of the samples with urine, seriously influence the results.
5. A simple apparatus for the determination of flow-elasticity in the field is described (the Oestroscope). An analysis of numerous determinations shows that the chances of a false diagnosis of oestrus by this means are small. The test is

easily carried out, takes no more than about 2 min. to complete and is therefore to be preferred to the other methods tried by us.

6. It is believed that the oestroscope technique should be of practical value to stock-breeders, especially for prediction of oestrus in cases where it is liable to be missed, and for its diagnosis in cases where ovulation is unaccompanied by external signs of 'heat' ('still-heat').

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