INVESTIGATION OF CERVICAL MUCUS AS AN INDICATOR OF OVARIAN ACTIVITY IN PIGS

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Summary. In eleven sows studied during the normal oestrous cycle, cervical mucus was most abundant and free-flowing during pro-oestrus and early oestrus and showed peak arborization 2 days before the onset of oestrus. Four gilts approaching puberty did not show typical arborization patterns until just before the second oestrus. In nineteen pregnant sows, only ten of 429 smears showed arborization. Fluctuating levels of reducing substances were found in cervical mucus throughout the cycle with a tendency for levels to be higher near oestrus. An increase in reducing substances preceded the pubertal oestrus in two of four gilts. Stained cervical smears from eleven cycles showed neutrophils and epithelial cells throughout the cycle. Large, vacuolated epithelial cells were predominant during metoestrus.

INTRODUCTION

The phenomenon variously described as crystallization or arborization of cervical mucus was discovered in women by Papanicolaou in 1946. He observed the crystalline pattern of leafy projections (which have since been compared with palm or fern leaves) after allowing mucus to dry unstained on a slide. Since then, the patterns have been the subject of much investigation by clinicians in human medicine (reviewed by Ullery, Livingston & Abou-Shabanah, 1959) and have been looked for and studied in cows, ewes, mares, bitches, goats, rabbits, donkeys, monkeys and sows (reviewed by Betteridge, 1961). Arborization is more pronounced during the follicular phase of the sexual cycle than during its luteal phase. A peak of arborization has been described as occurring at the onset of oestrus in cows (Alliston, Patterson & Ulberg, 1958) and in ewes (Raeside & McDonald, 1959). In the sow, Campos de Paz & da Costa Lima (1953) recorded a type of crystallization in the cervical mucus of one sow examined during the heat period. Coluzzi & Battistacci (1953) reported negative findings in three sows, and Polge (1960) has stated that the phenomenon has not been observed in sows. Nishikawa & Harada (1954) detected crystals in smears of sows' vaginal mucus.

Birnberg, Kurzrok & Laufer (1958) demonstrated an increase in level of

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reducing substances in the cervical mucus of women at ovulation. In their early work, they used ‘Tes-Tape’, an indicator paper that makes use of the Keston two-enzyme system (Keston, 1956) for the detection of glucose. Their later studies were made with ‘Ovustest’, the same enzyme system in a slightly more sensitive form. Similar glucose indicators in tape form are ‘Fertility Tape’, the use of which in women is described by Doyle, Ewers & Sapit (1960), and ‘Clinistix’, which has been used clinically by Harvey, Linn & Jackson (1960). In veterinary medicine, Bane & Rajakoski (1961) used both ‘Tes-Tape’ and ‘Clinistix’ to test the cervical mucus of cattle, but could demonstrate no definite cyclical variations in its glucose content. Polge (1960) has similarly rejected the use of ‘Clinistix’ on sows’ cervical mucus for detecting ovulation because positive reactions were found throughout the oestrous cycle.

Despite the extensive literature on the use of the exfoliative cytology of the cervix in women for the diagnosis of neoplasia, no reports of its investigation as a method for the assessment of ovarian activity in any species have been found. Several workers have studied the cellular content of the vaginal smear of the sow but have been unable to relate observed changes to specific phases of the oestrous cycle (Zupp, 1924; Altmann, 1941; Jaczewski, 1954; Schmidt & Bretschneider, 1954).

The present study was undertaken to evaluate the use of cervical mucus in assessing the endocrine status of non-pregnant, pregnant and prepubertal pigs.

MATERIALS AND METHODS

Cervical mucus of the normal oestrous cycle was examined for amount and consistency, arborization and content of reducing substances in two trials (1 and 2). The cytology of the cervical smear was investigated in material from Trial 2. Eleven sows were used, ten Large White and one Tamworth. All were between 1 and 3 years of age, and nine were known to have had litters. Oestrus was defined by the sows’ acceptance of mounting by a vasectomized boar. In Trial 1, daily examination of cervical mucus of five sows was made for at least one complete oestrous cycle covering eleven periods of heat. A sixth sow failed to show oestrus. In Trial 2, five animals were each examined over a complete oestrous cycle and a sixth sow daily for 2 weeks. This covered a further eleven periods of heat.

Four Large White gilts were similarly examined over two periods before the onset of puberty. Period 1 commenced approximately 12 weeks before the onset of puberty and lasted for 6 weeks. Period 2 began 4 weeks before the pubertal oestrus and lasted approximately 8 weeks, thus including the first oestrous cycle of each gilt. Examinations were daily except for the omission of a total of 7 days from Period 1 and 8 from Period 2.

Nineteen Large White females were examined for arborization at various stages of pregnancy. Nine of these were pregnant for the first time. Special

* Eli Lilly & Co Ltd, Greenfield, Indiana.
† Fertility Testor Co., Ottawa, Illinois.
‡ Ames Company.
emphasis was placed on the 3rd to 7th weeks of pregnancy, seven females being examined almost daily over that period.

All animals were housed in a piggery throughout the year and were fed a proprietary dry sow ration.

To take samples of cervical mucus, the sow was guided into an adjustable cattle-chute* but was not forcibly restrained. The vulva was examined for signs of swelling or redness, washed with disinfectant solution,† and then dried. The barrel of a ‘Coldlitc’ sigmoidoscope (13½ in. long × ½ in. diameter) was lubricated with antiseptic cream‡ and inserted as far as possible into the vagina. Removing the plunger and switching on the lamp then revealed the rugae of the cervix around the anterior end of the speculum. A sample of the mucus from this region was taken by means of a 14-in. glass rod, assessed visually for quantity (sparse, moderate or copious) and viscosity (thin or viscid) and left to air-dry on a clean microscope slide. The procedure was later modified slightly by wiping the exposed cervix with a surgical gauze sponge introduced on a metal swab carrier before taking the sample of mucus. This was in order to remove any vaginal contents that might have been pushed forward by the speculum. A separate glass rod was used to take each sample. In Trial 1 of the study of the normal oestrous cycle, and in the examination of pregnant animals, the rods were prepared by washing in detergent, rinsing in distilled water, drying and protecting the sampling ends from contamination before use by covering them with paper tissues secured by rubber bands. In remaining studies, the cervical mucus smears were made off the rods used to carry ‘Tes-Tape’ indicator paper. These were similarly washed and rinsed, and kept in a clean, covered tray until required. The indicator paper and rubber band attaching it to the rod had been found not to affect the ability of the mucus to show arborization. The barrel and plunger of the speculum were washed in a pail of the disinfectant solution after each examination and thoroughly dried with paper tissues before lubrication in readiness for the succeeding animal.

The cervical smears were examined microscopically with a low power objective and reduced illumination after they had completely dried, in the majority of cases within 8 hr of taking the sample but occasionally on the following day. Each smear was assessed for cellularity and arborization. The arborization, or palm-leaf formation (PL) was subjectively rated as follows:

- **Negative:** no arborization detectable.
- **Weakly positive:** very occasional and scattered patterns.
- **Positive:** occasional but well-formed patterns or patterns seen in most fields.
- **Strongly positive:** well-formed patterns seen in most fields.

The cellularity of the smear was classified as slight, moderate or great.

In testing for reducing substances, two proprietary indicators for glucose (‘Fertility Tape’ and ‘Tes-Tape’) were used in Trial 1. These indicator tapes were attached to glass rods by means of sections of rubber tubing. The in-

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* Ranger Western, Delphi, Indiana.
† 'Kem-25', Kem-San Products Ltd, Oakville, Ontario.
‡ 'Hibitane': Ayerst, McKenna & Harrison, Montreal.
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dicator rods were introduced into the cervix through the speculum after a sample of mucus had been collected. 'Fertility Tape' was first held in position for 90 sec followed by 'Tes-Tape' which was withdrawn after 30 sec. The times of insertion were selected after noting that, in women, Birnberg et al. (1958) used 30 sec for 'Tes-Tape' whereas Doyle et al. (1960) inserted 'Fertility Tape' for 3 min — an impractically long time in sows. Only 'Tes-Tape' was used in Trial 2 and was held in the cervix for 60 sec, the longer time being used in view of the 5-min period for which Birnberg et al. inserted 'Ovutest' in women. The rods were rotated periodically in order to soak the tapes with mucus as thoroughly as possible. After withdrawal, the deepest colour changes developing within 5 min were noted and, in the case of 'Tes-Tape', compared with a quantitative colour chart for glucose concentration. The colour reaction produced was scored as follows:

<table>
<thead>
<tr>
<th>Tes-Tape</th>
<th>Fertility Tape</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No change from yellow</td>
</tr>
<tr>
<td>*</td>
<td>Few patches of light green</td>
</tr>
<tr>
<td>**</td>
<td>Few patches of dark green (corresponding to 0.1% on glucose colour chart) or over-all light green</td>
</tr>
<tr>
<td>***</td>
<td>Over-all dark green</td>
</tr>
<tr>
<td>****</td>
<td>Over-all very dark green (0.25% glucose on colour chart)</td>
</tr>
<tr>
<td></td>
<td>No change from pink</td>
</tr>
<tr>
<td></td>
<td>Few patches of light blue</td>
</tr>
<tr>
<td></td>
<td>Few patches of dark blue or over-all light blue</td>
</tr>
<tr>
<td></td>
<td>Over-all dark blue</td>
</tr>
<tr>
<td></td>
<td>Over-all very dark blue</td>
</tr>
</tbody>
</table>

The smears of cervical mucus from Trial 2 were stained between 2 and 3 months later according to the method of Papanicolaou (1942) with the EA 36 stain. The smears had not been especially fixed but were air-dry and had been stored at room temperature. The stained smears, mounted in 'Technicon',* were examined under high power (×40 objective) and assessed for relative frequency of different cell types.

**Table 1**

DISTRIBUTION OF CERVICAL MUCUS TYPES ON THE BASIS OF APPEARANCE IN PL-POSITIVE AND PL-NEGATIVE SMEARS

<table>
<thead>
<tr>
<th>Amount:</th>
<th>Sparse</th>
<th>Moderate</th>
<th>Copious</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Viscid</td>
<td>Thic</td>
<td>Viscid</td>
</tr>
<tr>
<td>Consistency:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PL-positive (%)</td>
<td>2.7</td>
<td>8.0</td>
<td>21.3</td>
</tr>
<tr>
<td>PL-negative (%)</td>
<td>4.7</td>
<td>15.2</td>
<td>16.2</td>
</tr>
<tr>
<td>4.0</td>
<td>37.3</td>
<td>5.1</td>
<td>1.6</td>
</tr>
</tbody>
</table>

**RESULTS**

AMOUNT AND CONSISTENCY OF CERVICAL MUCUS

Three to 5 days before the 1st day of oestrus, cervical mucus increased markedly in amount and usually became thin and watery in consistency. It remained

* Technicon Co., Montreal.
like this during the first day or two of most heat periods but towards the end of oestrus or immediately afterwards it became very sparse and usually more viscid until the next pro-oestrus. These findings from Trials 1 and 2 of the normal oestrous cycle are shown in Text-fig. 1 and Table 1.

**ARBORIZATION OF CERVICAL MUCUS**

Arborization of cervical mucus was seen in a variety of forms (Pl. 1, Figs. 1 to 4) and no attempt was made to distinguish between them qualitatively.

Positive smears tended to appear during pro-oestrus. In Text-fig. 2 and Table 2, results from Trials 1 and 2 have been grouped and related to the 1st day of oestrus. Only those animals that were examined every day from at least 6 days before the 1st day of oestrus until at least 3 days after its onset have been included. The duration of arborization in cycles from Trial 1 ranged from 1 to 7 days with a mean of 3·4 days. In Trial 2, arborization was found over periods ranging from 1 to 12 days with a mean of 6·4 days. This extension of the time of occurrence is ascribable to the long duration of patterns in the cycles of two of the six animals examined (58P and 59P) and the presence of a weak pattern during the luteal phase of a third animal (390P). Thus, seven of the ten sows in Trials 1 and 2 showed arborization for from 1 to 7 days during an 8-day period commencing 3 days before oestrus. A peak occurrence was found 2 days before oestrus when seventeen of twenty smears were positive (Text-fig. 3). By arbitrarily allotting scores to the smears (0 for negative, 1 for weakly positive, 2 for positive and 3 for strongly positive) the ‘strength’ of arborization was also found to be greatest 2 days before oestrus (Text-fig. 4). Positive patterns were seen in mucus of various consistencies and quantities but most (over 70%) were found in thin mucus (Table 1). The sow that failed to come into oestrus gave PL-positive smears on 2 of the 43 days on which she was examined.
### Table 2

**Arborization of Cervical Mucus During the Oestrous Cycle of the Sow**

<table>
<thead>
<tr>
<th>Days relative to first day of oestrus</th>
<th>-13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1 0* +1 +2 +3 +4 +5 +6 +7 +8 +9 +10</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. smears examined</td>
<td>7 8 8 13 14 15 20 20 20 20 20 20 18 19 17 17 14 12 8</td>
</tr>
<tr>
<td>No. positive smears</td>
<td>0 0 0 1 0 1 2 3 2 1 8 17 11 5 7 3 2 2 1 0 1 0 0 0</td>
</tr>
<tr>
<td>Score†</td>
<td>0 0 0 1 0 1 0 2 3 5 3 1 16 45 23 12 14 5 3 3 2 0 1 0 0 0</td>
</tr>
</tbody>
</table>

* First day of oestrus.  
† See Text-fig. 2.
All four prepubertal gilts showed star-shaped 'arborization' patterns (Pl. 1, Fig. 5) during the first 9 days of Period 1, but they were not seen during the remainder of the trial except in one smear. Though these smears were classified as PL-positive, the patterns were of a type that had not been seen in normally cycling sows. Similar patterns were seen in two of the gilts during Period 2, 2 days before the onset of the pubertal oestrus in one case, and 11 days before its onset in the other. Arborization patterns preceding the second oestrus in all gilts, however, were like those formed during the oestrous cycles of adult sows and reached a similar peak 2 days before the 1st day of heat.

The incidence of arborization of cervical mucus during pregnancy was low. Between the 13th and 60th days of gestation, 216 samples from ten females (mean of 4.5 females per day) were all negative for arborization. From Day 61 to Day 90 of gestation, 103 samples from eleven females (mean 3.4 per day) included two showing arborization and six with weak pattern formation. These eight samples were from five different animals. From Day 91 until term, 110 samples from eight females (mean 4.4 per day) included a single positive smear from each of two animals.

![Text-fig. 2. Arborization of cervical mucus during the oestrous cycle of the sow.](image-url)
Cervical mucus in pigs

REDUCING SUBSTANCES IN CERVICAL MUCUS

The thoroughness with which the indicator tapes were soaked in cervical mucus varied with its consistency. When mucus was sparse, the tapes remained at least partially dry and colour changes were in patches. Green colour responses with ‘Tes-Tape’ usually intensified in the 1st min or so after removal of the rod from the cervix but faded between 5 and 10 min later. Blue colour responses with ‘Fertility Tape’ intensified after withdrawal. In Trial 1, no consistent sign of cyclic variation was shown by ‘Fertility Tape’, but ‘Tes-Tape’ indicated higher levels of reducing substances for a few days around the beginning of heat (Text-fig. 5). The degree of the rise and the time of the cycle at which it began were variable. In six of the eleven heat periods in Trial 1, a sharp drop in level of reducing substances was indicated by ‘Tes-Tape’ before the end of heat. These results were not confirmed in Trial 2 for, out of the twelve periods of oestrus examined, only six conspicuous rises in amounts of reducing substances were detected by ‘Tes-Tape’ (Text-fig. 6). Five of these rises occurred between 2 and 5 days before the 1st day of heat and one was detected 3 days after its onset.
Text-fig. 5. Reducing substances in cervical mucus during the oestrous cycle of the sow (Trial 1).

Text-fig. 6. Reducing substances in cervical mucus during the oestrous cycle of the sow (Trial 2).
‘Tes-Tape’ indicated that levels of reducing substances in the cervical mucus of the prepuberal gilts tended to be higher during the 1st week of Period 1 than over the remaining weeks, but showed no evidence of cyclic variation. During Period 2, noticeably higher levels of reducing substances were detected 1 day before the 1st day of the pubertal oestrous of one gilt (460R) and 2 days before its onset in another (487R). No such rises immediately before the pubertal oestrous were seen in the two other gilts. Earlier in Period 2, there were days for each gilt when cervical mucus had higher than usual levels of reducing substances. The days on which this occurred were as follows (negative numbers indicating ‘before the 1st day of oestrous’):

Gilt 448R, Day -21;
Gilt 460R, Days -25 to -23;
Gilt 486R, Day -20;

During the first complete oestrous cycle, the best defined rises in levels of reducing substances before oestrous were again in gilts 460R and 487R, 3 to 5 days before oestrous in the former animal and on the 2nd and 4th days before, in the latter.

**CYTOLOGY OF CERVICAL SMEARS**

Two cell types were readily recognizable in the unstained smears as they were examined for arborization; neutrophils (which appeared as small, round, dark bodies) and epithelial cells (larger than the neutrophils and with a recognizable nucleus). Arborization was most common in smears classified as being ‘slightly’ cellular. In almost all PL-positive smears, most of the cells were neutrophils. Near oestrous, these cells were often arranged in a characteristic manner, like beads in a necklace.

In smears stained with Papanicolaou’s stain, the appearance of the epithelial cells varied widely from pyknotic nuclei surrounded by a very narrow rim of cytoplasm to large cells with vacuolated eosinophilic cytoplasm and pale-staining nuclei (Pl. 1, Figs. 6 and 7). The presence of numerous large epithelial cells was a cyclic occurrence, being the dominant feature of smears made during metoestrus in each of 11 oestrous cycles. They appeared either at the beginning of oestrus (Day 0) or between then and Day 2 of the cycle and remained numerous for between 2 and 8 days. It was not possible to time their persistence accurately because smears during that stage of the cycle were often very sparse. Most epithelial cells found during the rest of the cycle were smaller and had small densely staining nuclei. Neutrophils were found in smears at all stages of the cycle. They were more numerous in the smears of some animals than in those of others, and, in eight of the eleven cycles, appeared to increase in numbers for between 4 and 9 days, beginning 1 to 4 days before the 1st day of oestrus.

**DISCUSSION**

The presence of mucus at the vulvar lips of the sow is a well known sign of oestrus. Altmann (1941) found it to be maximal late in oestrus, whereas present
work shows that the cervical mucus is abundant and free flowing before oestrus and tends to become sparse towards the end of the heat period.

Arborization patterns are most frequently seen 2 days before oestrus, and are often absent from the cervical mucus of sows actually in heat, which may account for some of the negative findings reported in the literature. It is interesting to note that urinary oestrogen excretion in the sow shows a sharp peak at the beginning of oestrus and sometimes even before its onset (Raeside, 1962). In spite of the peak incidence and intensity of arborization, the variability in the time of onset of the phenomenon precludes its use for timing the beginning of oestrus or ovulation. Nor does the detection of crystal patterns definitely foreshadow oestrus since one sow provided positive smears during anoestrus.

Arborization comparable to that seen in mature sows was not observed in any of the four gilts examined before puberty. The patterns seen during the first few days of the initial examination may have been a property of fluids that had accumulated before repeated examinations were begun, for they were seen only sporadically after the first 9 days of Period I. Thus the cyclic ovarian changes that occur in gilts before puberty (Wetli, 1942) are not reflected in arborization changes of cervical mucus. In this connexion, however, the phenomenon has probably indicated ‘silent’ heats in ewes (McDonald & Raeside, 1956).

The low incidence of arborization of cervical mucus in pregnant sows is consistent with results reported in women, cows and ewes. The absence of the phenomenon during the 3rd to 7th weeks of pregnancy in the seven females examined suggests a possible aid to pregnancy diagnosis in swine: daily examination of cervical mucus during the 3rd or 6th week after service should reveal arborization if the animal has not conceived and is returning to oestrus. This, of course, would be more arduous and no more reliable than merely observing the animals for the more usual signs of oestrus.

‘Tes-Tape’ revealed a tendency for the concentration of reducing substances in cervical mucus to be higher during pro-oestrus and oestrus in most animals tested but the time-relationship of the higher levels to oestrus was inconsistent. ‘Fertility Tape’ readings were even more erratic. In the sow, therefore, neither indicator could be used to detect ovulation as it has been claimed they can in women. The higher readings recorded in the gilts between 3 and 4 weeks before puberty may possibly have been related to ovarian activity.

The increase in the numerical proportion of the epithelial cells in cervical smears after oestrus is comparable to the change in vaginal smears recorded by Wilson (1926). The vacuolation of these cells is also similar to that illustrated by him, yet Altmann (1941) found the epithelial cell content of vaginal smears from sows to fluctuate without a clear-cut connexion with oestrus. Both authors, however, report a decrease in leucocytes during metoestrus. This was not a constant finding in the cervical smears, for the pre-oestrus increase in leucocytes observed in most cycles often extended into metoestrus. As had been found for the vaginal smear by earlier workers, the cytology of the cervical smear in this study did not provide a very accurate means of assessing ovarian function in pigs, and certainly could not be used to predict the occurrence of either oestrus or ovulation.
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REFERENCES


EXPLANATION OF PLATE 1

Smeared of cervical mucus from pigs

Figs. 1 to 4. Types of arborization patterns seen in the cervical mucus of sows during the normal oestrous cycle.

Fig. 5. Star-shaped patterns in the cervical mucus of a prepubertal gilt.

Fig. 6. Cervical smear on Day 2 of the oestrous cycle.

Fig. 7. Cervical smear on Day 10 of the oestrous cycle.

(Figs. 1 to 5, unstained ×94; Figs. 6 and 7, Papanicolaou's EA 36 stain, ×375.)