Crystallization patterns in anterior vaginal fluid from bitches in oestrus

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Summary. Bitches exhibited a characteristic arborization pattern of the fluid from the anterior vagina during pro-oestrus and oestrus. These changes were monitored together with conventional vaginal cytology and plasma oestrogen and progesterone concentrations. A classical ferning pattern, similar to that seen in bovine cervical mucus at oestrus, occurred after the peak in plasma oestrogen concentrations. Ferning was most intense after the second peak of cornification of vaginal epithelial cells. It is suggested that a ‘Ferning Index’, when combined with conventional vaginal cytology, can be of use in determining the optimum mating time in the bitch.

Keywords: vaginal fluid; crystallization; bitch

Introduction

The phenomenon described as crystallization or arborization of human cervical mucus, which occurs when mucus is allowed to dry unstained on a microscope slide, was discovered by Papanicolaou (1946). Subsequently Garm & Skjerven (1952) studied crystallization of cervical mucus in cattle and noted that the fern-like crystals appeared during oestrus and disappeared in the luteal phase of the cycle. Many workers have related ferning patterns to the stage of the oestrous cycle, noting that maximal arborization occurred at the onset of oestrus (Bone, 1954; Alliston et al., 1958; Fallon & Crofts, 1959; Abusinein, 1962; Noonan et al., 1975; Grobbelaar & Kay, 1985). The changes in the physical properties of the secretions have been attributed to changes in chemical composition (Roychoudhury & Razden, 1965). Aboul-ela et al. (1983) related ferning of mucus, electrolyte composition and electrical resistance to hormone concentrations. Watson (1939) influenced the secretion of mucus by using injected oestrogens and Glover (1960) showed that in cows oestrogens stimulated watery mucus similar to that seen during oestrus whilst progesterone stimulated thick mucus similar to that seen during dioestrus or pregnancy.

Arborization of cervical mucus has been investigated in many species (Betteridge & Raeside, 1962) but there are no detailed reports concerning its occurrence in the bitch.

Materials and Methods

Six anoestrous bitches were examined daily and vaginal cytology was studied twice weekly until the onset of pro-oestrus (starting with the first signs of vulval oedema and blood tinged discharge); anterior vaginal fluid was then collected daily. Eleven other bitches, presented for infertility investigation, were examined as frequently as possible.

Fluid was collected using an adaptation of the method of Allen & Dagnall (1982); a sterile insemination pipette was introduced into the anterior vagina, carefully directing the pipette upwards through the vestibule and then horizontally as far as possible. A 5-ml syringe attached by a small piece of rubber tubing was used to apply suction. A sample of the fluid collected was placed onto a tilted glass microscope slide to allow it to spread and a thin smear was made on a second slide; both were allowed to dry at room temperature.
The first slide was examined microscopically at ×400 magnification for arborization. Several ferning patterns had been previously identified, photographed and divided into five classes from minor ferning (with short stems and irregular scattered stellate patterns) to bold ferning (with long stems and clear venation and subvenation).

Ferning was compared with the photographic records and given a score of 0–5 (Figs 1a–e). A subjective assessment of the surface area covered by arborization was made, and a score given according to the following criteria; up to 1% cover (score 1), 1–5% cover (score 2), 5–10% cover (score 3), 10–20% cover (score 4) and over 20% cover (score 5). The fern score and cover score were added to give the 'Ferning Index'.

The smear was stained with M & D Diff-Quik (American Hospital Suppliers, Didcot, Oxon, UK) and cytology was examined. The percentage of cornified epithelial cells was calculated as the 'Cornification Index' (van der Holst & Best, 1976). Heparinized blood samples were collected from the jugular vein for oestrogen and progesteron determination. Total unconjugated oestrogens were measured in the plasma by radioimmunoassay as described by Challis et al. (1971), using an antiserum which mainly cross-reacted with oestradiol-17β (100%), 6a-hydroxyoestradiol (82%), oestradiol-17α (5–3%), oestrone (4.8%) and oestradiol (1.3%). The assay detection limit was 5 pg/ml and the coefficient of variation at 25 pg/ml was 6.8%, and at 250 pg/ml was 19.3%. Progestagens were measured by the radioimmunoassay method described by Cockrill & Allen (1978). The antiserum cross-reacted mainly with progesterone (100%), 11α-hydroxyprogesterone (85%), 17α-hydroxyprogesterone (12.5%), 5β-pregnane-3,20-dione (12.5%), 5α-pregnane-3,20-dione (3%), 5β-pregnane-3β-ol-20-one (1.7%), 11-deoxycorticosterone (1.1%) and 5α-pregnane-3β-ol-20-one (1%). The assay detection limit was 0.05 ng/ml and the coefficient of variation at 0.63 ng/ml was 35%, and at 1.79 ng/ml was 16.8%.

Results

Figure 2 demonstrates the changes in the Cornification Index, the Ferning Index and plasma steroid concentrations during the oestrous periods of the 6 bitches. The calculated ovulation days are indicated for Bitches B, D and F. This was assumed to be 63 days before parturition, i.e. 65 days from the LH surge to parturition (Concannon et al., 1983) with a 2-day interval between the LH surge to ovulation (Concannon et al., 1977).

Bitches A, B and D exhibited a maximum fern score of 5, Bitch C a fern score of 4, and Bitches E and F a score of 3. Ferning patterns were identified in 9 of the 11 clinical cases. These were always maximal following the peak of cornification; in 3 instances pregnancy and parturition allowed the calculation of ovulation dates, which occurred within the peak of ferning. In the 2 instances when ferning was not present, vaginal cytology demonstrated few cornified epithelial cells and many polymorphonuclear leucocytes; it was concluded that the bitches were in late oestrus when ferning should not have been present anyway.

Discussion

Plasma oestrogen concentrations increase during pro-oestrus and reach a peak just before or at the start of oestrus (Bell et al., 1971). This preovulatory oestrogen surge is probably responsible for stimulating the release of luteinizing hormone (LH) which precedes ovulation by about 2 days (Phemister et al., 1973; Concannon et al., 1977). During this period there is a slow increase in the plasma progesterone concentrations from early pro-oestrus to late oestrus. The hormonal changes exhibited in the 6 bitches in this study follow this pattern. The intensity of ferning was not as great as in other species especially during early oestrus (fern scores 1–3), possibly due to dilution with other secretions.

Several authors have stressed that in the cow cervical and not vaginal mucus should be collected since vaginal mucus is not such a good indicator of reproductive status (Bone, 1954; Abusineina, 1962; Noonan et al., 1975). It is not possible to be as selective in the bitch which may explain the
Fig. 2. Changes during oestrus in the Cornification Index, Ferning Index and plasma oestrogen and progesterone concentrations for Bitches A–F. Arrows indicate the calculated day of ovulation.

variation seen between animals. Further work is being undertaken to elucidate the site of production of the mucus. Occasionally during early pro-oestrus aspiration was not successful and had to be repeated. Zondek & Rozin (1954) have suggested that blood inhibits ferning of mucus, but this is not the case in women (Williams, 1964) or, as the present study demonstrates, in the bitch. In each bitch the greatest ferning occurred after the second rise in the Cornification Index and between 1 and 5 days after maximal oestrogen concentrations. This would correspond to the period just before or at ovulation when follicles are becoming luteinized.

Van der Holst & Best (1976) found that the highest pregnancy rates occurred when bitches were mated at the peak of cornification. In some bitches the Cornification Index only reaches 60%, and many bitches have two cornification peaks (van der Holst & Best, 1976) which may cause confusion and the optimum mating time may be missed. The combined use of vaginal cytology and ferning patterns helps to distinguish the relevant cornification peak, and the technique has proved to be useful, especially in bitches with prolonged periods of pro-oestrus.

We thank A. Young for zootechnical assistance; D. Porter for performing the hormone assays; and D. Gunn for producing the figures. G.C.W.E. was in receipt of the Thomas Brown Research Fellowship.
References


Received 23 September 1988