CERVICAL MUCUS ARBORIZATION : A PRELIMINARY STUDY OF ITS USE IN ASSESSING OVARIAN HORMONE LEVELS IN THE EWE

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I INTRODUCTION

Many of the gross physiologicial processes affecting reproduction in animals have been elucidated. The availability of the ovarian hormones in relatively pure form has further allowed the confirmation of their role. It is apparent that there is general agreement and confidence in the findings of much of this work, as is shown by the increasing application of steroids to animal production. However, in the main the investigations so far reported have been of a qualitative nature only. It is thus apparent that for a thorough understanding of the basic mechanisms controlling reproduction it will be necessary also to study in detail the quantitative changes in hormone levels and their associated effects.

Progress in the investigation of the quantitative changes has been slow, owing to the lack of suitable techniques of study. A number of criteria have however been utilized to study ovarian activity and ovarian hormone levels. Unlike other large species, palpation of the ovaries per rectum is not possible with the ewe. Oestrous behaviour and visual observation of the ovaries after laparotomy thus remain the methods for assessment of ovarian activity. Biologicial assay of body fluids and excretory products using small animals has been somewhat successful. To avoid the attendant problems of the bioassay chemical methods are being developed but as yet

have met with limited success. In addition, biological endpoints obtained from within have been used. The characteristic vaginal smear changes found in the ewe are favoured by some workers, although there is not entire agreement on the application of this method. So far no work has been reported for the ewe utilizing the rheological properties or the arborization phenomenon of cervical mucus. However, the results obtained with other species suggest their possibilities. It thus seemed apparent that the investigation of one of these phenomena would be worthwhile.

Cervical mucus arborization, first reported by Papanicolaou (1945) and later used by Zondek (1954) as a biological test in the human was suggested as an end-point for determining ovarian activity. The object of this study was to investigate cervical mucus arborization and to demonstrate its application as a method for study of ovarian function and hormone levels. Thus, as will be described presently, a preliminary study of the normal occurrence of this phenomenon in the entire ewe was made. In addition, cervical mucus along with the criteria of oestrous behaviour and vaginal smear changes was used to study some effects of progesterone and oestrogen injected into castrate ewes. The results of these trials were subsequently related to studies made with entire ewes.

II REVIEW OF LITERATURE

A. Cervix

1. Morphology

"The cervix or neck of the uterus consists of a powerful sphincter like segment of the genital tract, serving to separate anatomically and physiologically, the uterus from the vagina. It is continuous anteriorly with the uterine body via the os uteri and posteriorly with the vagina by the os externum which leads into the ventral floor of the vagina" (Sisson, 1953).

The comparative anatomy of this organ has been described for the ewe, sow and mare (Trautman, 1917). Williams (1917) has described that for the cow.

The cervix uteri of the ewe is remarkable for the great length and complexity of its lumen, as shown by the diagrams of Marshall and Hammond, (1937). The dimensions of the cervix have been stated by Grant (1933), Cloete (1939), and Sisson (1953). During periods other than at or close to parturition, the length of the cervix is 5 cm. approximately. The lumen of the cervix was greatly constricted by annular or tongue-like folds and ridges projecting inwards. These interleaved with one another when the cervix was contracted and completely blocked the passage. Because of this it was difficult to insert any instrument very far into the cervical canal of the ewe (Walton, 1933; Grant, 1934; Gunn, 1936; Inkster, 1956). However Grant (1934) did note that relaxation of the cervix was evident at oestrus. Four or five folds were effective in closing the cervix in the

ewe (Gunn, 1936) and the cow (Hammond, 1927). With the human and mare these folds were less apparent, while the tongue-like projections were absent.

The complexity of the external orifice of the cervic (os externum) was observed by Grant (1934), who noted one or more muscular folds to constitute the os externum. Dunn (1955) studied the vaginal - cervical junction, before and after slaughter. It was found that the structure of the os externum became more complex, as the number of parturitions increased. The annular folds appeared to be split by pregnancy and "tags" of musculature projected out through the canal opening. The passage or canal was more difficult to locate in aged ewes which had had more pregnancies than in maiden ewes. Dunn found that the os externum could be located in 100 per cent of maiden ewes, but in only 70 per cent of older sheep.

2. Histology

a. Sexual season

Casida and McKenzie (1932) Grant (1934), Cole and Miller (1935), and McKenzie and Terrill (1937) found the mid-cervical epithelium region to secrete mucus throughout the cycle. However, most mucus appeared to be produced and stored during diestrus and later liberated at proestrus and oestrus. It was evident that the changes in height of the epithelial layer was closely associated with the amount of mucus in the cells. Thus a "spent appearance" during the late luteal and follicular

phases was observed (McKenzie and Terrill, 1937).

b. Anoestrus and pregnancy

Grant (1934) detected mucus in the epithlial cells throughout anoestrus but noted that active secretion began approximately six weeks before the commencement of the breeding season. Cole and Miller (1935) found that mid anoestrus was characterized by a lower cell height of the superficial layer, less active underlying epithelial cells and reduced complexity of the cervical glands.

The changes occurring during pregnancy have been noted by Grant (1934). A tenacious, stringy mucus was secreted during most of pregnancy. Since little escaped into the vagina a cervical mucous plug was formed, which flattened and disorganized the epithelial cells.

B. Characteristics of Cervical Secretion

1. Origin and amount of cervical mucus

Pommerenke and Viergiver (1946 a) investigated the origin of cervical mucus in women. During pregnancy, the chorion, decidua and amnion effectively sealed the cervical canal from the uterine cavity, hence the mucus was likely to be entirely of cervical origin. These workers could not demonstrate that supracervical hysterectomy resulted in a lower output of mucus, than from intact non-pregnant women. However, they considered that for non-pregnant women the evidence was insufficient to determine the origin of the cervical secretion.

In the human the cervical canal was relatively open and mucus was aspirated easily. Abarbanel (1946 b) determined the volume and noted a relationship with the menstrual cycle. Mucus was found in greatest quantity near the time of ovulation (approximately the middle of the menstrual cycle), while a greatly reduced amount was apparent at other times. Similar findings have been reported by Pommerenke (1946), Pommerenke and Viergiver (1946 a, b,), and Zondek and Rozin (1954).

The difficulty in obtaining all of the mucus contained in the cervix of the cow and ewe appears to have prevented accurate measurement of the volume in the living animal. However, Woodman and Hammond (1925) with slaughter material from cows, noted a

gradual increase in amount of mucus until oestrus, but a decrease occurred just after heat.

Pregnancy was marked by a rise in total volume, over that of the oestrous cycle. Reark and Herman (1950) similarly found the greatest volume of mucus within the first three hours of oestrus in the live animal.

Grant (1934) reported the absence of mucus secreting glands in the vagina of the ewe. The mucus volume he measured originally came from the cervix. Further, it was observed that the volume of mucus secreted followed a cyclic pattern. A copious flow commenced at proestrus and continued during oestrus but this was greatly diminished during the rest of the cycle.

In conclusion it is apparent that considerable variation in the amount of cervical mucus secreted does exist between species. The findings of Viergiver and Pommerenke (1944), and Pommerenke and Viergiver (1946 a) with intact and hysterectomised women shows that a large variation also exists within a species.

2. Chemical properties

Detailed examination of human cervical mucus has revealed that the chemical composition varied according to the stage of reproduction (Pommerenke and Viergiver, 1946 b; Viergiver and Pommerenke, 1947; Atkinson et. al., 1948; Pederson and Pommerenke, 1950; Bergmann and

Werner, 1951; Breckenridge and Pommerenke, 1951; Shettels, 1951; Shettels et. al., 1951).

Thus during the interrenestrum, the water content rose while the concentration of carbohydrate, amino acids, cholesterol and lipid phosphorus decreased.

Changes in composition of cervical mucus from the cow during the oestrous cycle and pregnancy have also been reported (Woodman and Hammond, 1925; Scott Blair et. al., 1941 b; Boyland, 1946). Thus the latter worker noted that cervical mucin present at oestrus was mainly a carbohydrate and possibly a mucopolysaccharide. However, during dioestrus and pregnancy both polysaccharide and protein were present.

The literature does not indicate any analysis made for mucus from the ewe.

3. Physical properties

a. Rheological

Woodman and Hammond (1925) have described the character of the cervical secretion of the cow. They noted the secretion to be extremely fluid at oestrus, but more viscous during mid-cycle. Pregnancy, was characterized by large amounts of a thick, tenacious, almost rubber-like mucus which effectively sealed the entrance to the cervix. This phenomenon was later proposed as the basis for a pregnancy test (Marshall and Hammond, 1937).

The rheological properties of human cervical secretion have been studied extensively. Scott Blair

et. al. (1941 a, b) and Clift (1945) also measured the flow elasticity* of cervical secretion from cows. Maximum values occurred near the time of ovulation, while much lower values were obtained during the rest of the cycle and in pregnancy. Urine contamination of the mucus caused erroneous values for viscosity measurements, but did not appear to affect flow elasticity. The additional characteristics of spinnbarkeit,** plasticity and tact have also been investigated for the human (Clift, 1945; Cohen et. al., 1952).

Further work (Clift et.al., 1950; Glover and Scott Blair, 1951; Clift and Hart, 1953; Glover and Scott Blair, 1953; Scott Blair, 1953) using mucus from cows and women, indicated that the variations in flow properties were closely correlated with the physiological changes of the cycle and pregnancy.

*When a column of fluid is being extruded from a capillary tube and the pressure is suddenly released the column will recoil. The amount of recoil is a measure of the flow elasticity of the fluid, (Scott Blair et. al., 1941 b).

**Spinnbarkeit - the capacity of liquids to be drawn into threads, (Clift, 1945).

b. Arborization phenomenon

Papanicolaou (1945, 1946) observed that cervical mucus collected from women near the time of ovulation "crystallised" and formed a typical arborization pattern* when allowed to dry on a microscope slide. Under the microscope, the smear had a striking pattern of flowers and leaves resembling fern or palm-leaves. Zondek (1954) described this as "fern" or palm leaf (PL) formation. However the type of pattern was dependent partly upon the thickness of mucus; arborization often failed with thin smears, whereas flower patterns and palm leaf patterns were found with thicker mucus.

Arborization has also been shown to be characteristic of cervical mucus from the cow (Garm and Skjerven, 1952; Colluzzi and Battistacci, 1953; Higaki and Awai, 1953; Bone, 1954; Fedrigo, 1955; & Lora 1955) and the ewe (Rseside, unpublished data, 1955).

i. Mechanism of arborization - Papanicolaou (1946) observed that the abundance of arborization coincided with increased cervical secretory activity and a change in viscosity of the fluid. However Zondek (1954) stated that arborization and elasticity of mucus were independent.

Rydberg (1948) suggested that the phenomenon was caused by crystals of sodium chloride. The characteristic

*Zondek (1954) has suggested that true crystals may not be involved in the phenomenon and preferred to term the process arborization.

forms which these assumed were thought to be dependent upon the presence of mucin-like substances secreted by the cervical glands. An intensive investigation reported by Zondek (1954) showed that arborization resulted when a protein complex was mixed with electrolytes and allowed to dry. Examination of many body fluids including nasal mucus, cerebrospinal fluid, follicular fluid, ovarian cystic fluid and that from the hydrosalpinx all showed arborization when completely dry. Detailed examination of the constituents of mucus showed that mucin was not the essential factor as suggested by Rydberg (1948). Thus tears which did not contain mucin or similar substances showed a typical PL reaction. Further, it was found that albumin, fibrinogen, and globulins, and the degradation products of protein (including peptones, dipeptides, tripeptides and polypeptides and certain amino acids), as well as monosaccharides and polysaccharides all showed arborization when mixed with electrolytes.

ii. Factors affecting arborization - In the literature several factors which were likely to influence the PL reaction have been discussed.

Electrolyte status: Landerstrom-Lang produced typical arborization by drying a mixture of egg albumin and 0.9 per cent sodium chloride. Zondek (1954) showed that neither the sodium or chloride ions were specific to the PL reaction, since potassium bromide also

facilitated arborization when mixed with a protein complex. Some salts failed to cause a PL reaction, notably calcium chloride, barium chloride, sodium bromide, potassium nitrate, sodium sulphate and sodium iodide.

The presence of electrolyte was essential to the arborization process since dialysis of cervical mucus inhibited arborization, whereas subsequent addition of electrolyte allowed PL formation (Zondek 1954). Further, it was noted thatcellular type cervical mucus (negative smear) from premenstrual, menstrual, postmenstrual, pregnant, menopqueal and centrate women developed arborization when electrolyte solution was added.

High concentrations of electrolyte were found to facilitate the PL reaction better than low concentrations (Zondek, 1954). However, arborization was still apparent even when low concentrations of both protein complex and electrolyte were present. Zondek concluded that for cervical mucus any protein or carbohydrate will produce arborization, provided a minimum concentration of electrolyte was present. The possibility that still unknown factors present in cervical mucus might be involved in the phenomenon, was also stressed.

Temperature: Papanicolaou (1945), Rydberg (1948), Roland (1952), Zondek (1954), Bone (1954), Zondek et. al., (1955) observed arborization after cervical mucus

was dried at air temperature. The atmospheric temperature was not stated. Campos da Paz (1953), and Zondek (1954), dried the smears in a flame and found arborization persistent even when the smear turned brown at high temperatures. No recordings appear to have been made of low temperature effects on arborization.

Foreign materials: Bone (1954) in a preliminary study of cervical mucus of the cow observed that the blood chlorides normally present in serum or tissue fluids failed to promote arborization. Zondek (1954) noted a similar condition with human cervical mucus. Further, he found that sperm and prostatic secretion inhibited the PL reaction. Similarly, boiled sperm prevented arborization and thus excluded the possibility that enzyme action was responsible.

No investigation of the mechanism by which these contaminants inhibit arborization has so far been reported. Zondek (1954) suggested an inhibition of arborization by a "mechanical effect" of the contaminants. It should be mentioned that this was in contrast to hormonal inhibition of arborization, to be discussed later.