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EXOGENOUS ENERGY SOURCES FOR SPERMATOZOA IN CERVICAL MUCUS OF THE COW AT OESTRUS

R. GIBBONS, K. COLLIS AND R. SELLWOOD

Agricultural Research Council,
Institute for Research on Animal Diseases, Compton, Newbury, Berkshire

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Bovine spermatozoa have been shown to metabolize exogenous substrates both aerobically and anaerobically (Mann, 1964). Fructose (Mann, 1946) and sorbitol (King & Mann, 1958) have been unequivocally identified in seminal plasma, but evidence for the presence of metabolizable substrates in the cervical mucus rests on much less secure foundations. A number of reports that glucose is present in cervical mucus have appeared and indeed Doyle (1958) proposed to use the presence of glucose in human cervical mucus as an index of ovulation. This test has not attained widespread acceptance although there is a general impression that the sugar is present (see e.g. Moghissi, 1973).

The original authority for the presence of glucose in bovine cervical mucus at oestrus is Olds & VanDemark (1957) who used, as others have done since, measurements of reducing value which are appropriate for deproteinized serum. Moreover, quite high values for fructose in this secretion have been reported apparently on the basis of the reaction of the mucus with orcinol in strong acid (El Naggar & Horvath, 1971). These non-specific methods are unsatisfactory and have been criticized by Mann (1973) who pointed to the need for specific enzymatic or chromatographic techniques to reappraise the position of cervical mucus with respect to the presence of substrates for sperm metabolism. Weed & Carrera (1970) report values up to 2 mg/ml in women.

R. A. Gibbons (unpublished observations) could not detect free glucose in bovine cervical mucus using glucose oxidase and Werner (1959) also reported that the amounts of free glucose in human cervical mucus were negligible.

In the investigations now reported, three samples of bovine mucus were used, of which two were collected from individual animals and were processed immediately and the third was from a pooled sample from fifteen cows which had been collected over a period and stored frozen until required. The latter sample was split into three aliquots of approximately 3 ml. To one of the aliquots, 150 μg each of fructose, sorbitol and glucose was added and to another 60 μg of the same three substances was included; this was done to confirm that mucus contained nothing that could inhibit the enzymatic reactions for the estimation of these sugars.

The five mucus samples were centrifuged at 115,000 ***g*** (av.) for 4½ hr. The resulting supernatants (0·2-ml aliquots) were analysed for glucose using hexokinase followed by glucose-6-phosphate dehydrogenase and NADP. Fructose was then estimated by the addition of phosphoglucose isomerase (Bergmeyer,

Bernt, Schmidt & Stork, 1970). Sorbitol was estimated on a separate aliquot using sorbitol dehydrogenase and NAD (Bergmeyer, Gruber & Gutmann, 1970). The reduction in NADP and NAD was measured using a Vitatron automatic u.v. spectrophotometer, type UFD system. Fructose could not be detected ($<0{\cdot}2$ μg/ml); glucose values ranged from undetectable to as high as 9 μg/ml in one individual sample. Sorbitol ranged between 2 and 5 μg/ml. All three compounds, when added to the mucus, were recovered quantitatively.

The supernatant fraction was shown by immunodiffusion in gel (Ouchterlony, 1964) to contain serum protein, and it seems rather remarkable that these serum proteins may be found in cervical mucus (Schumacher, 1972) whilst the low molecular weight serum component, glucose, is not. The straightforward interpretation of the presence of serum proteins in mucous secretions as being due to transudation or leakage needs some amendment.

The discrepancy between the estimates of fructose and glucose by chemical and by enzymatic methods is easily understood. Mucus is rich in epithelial glycoprotein, which contains the very alkali-labile seryl or threonyl glycoside structure (Anderson & co-authors, 1964). Methods for estimating reducing sugars are carried out under alkaline conditions, which cause sufficient cleavage and release of reducing groups from epithelial glycoprotein to give it some apparent reducing value; about 2 to 3% of that of glucose for the method of Nelson (1944). Again, the orcinol method, though more sensitive to ketoses than aldoses, will give appreciable colour with the galactose, fucose and neuraminic acid of epithelial glycoprotein (Brückner, 1955).

It is concluded that neither of the two sugars studied nor sorbitol is an energy source for spermatozoa in the bovine species once the gametes have left the seminal plasma. This occurs quite quickly after ejaculation. Mucus which has been dialysed against buffered saline will support sperm activity *in vitro* at 37°C as effectively as the untreated mucus (Mattner, 1966). While this, being an *in-vitro* experiment, is not conclusive, it would clearly be desirable to see how human spermatozoa behave in human cervical mucus similarly treated. Indeed, human mucus should be re-examined for substrates available as energy sources using more critical methods of analysis. The values reported for glucose by Weed & Carrera (1970), who used a ferricyanide reduction method, appear unrealistically high.

If, as appears possible, conditions in the female genital tract are sufficiently aerobic for spermatozoa to utilize endogenous reserves (mainly phospholipid—Hartree & Mann, 1961), these may suffice to maintain adequate sperm motility for fertilization to be achieved.

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