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THE MICROBIOLOGY OF BOVINE SEMEN  
AND  
THE ANTIMICROBIAL ACTIVITY OF BOVINE SEMINAL PLASMA

A thesis presented in partial fulfilment  
of the requirements for the degree of  
Doctor of Philosophy in Microbiology at  
Massey University.

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ABSTRACT

Microbial populations in semen from 18 bulls used for artificial breeding, varied between 30 and 560,000 organisms/cm<sup>3</sup>, with a mean of 3,300/cm<sup>3</sup>. Wide day to day fluctuations occurred in the microbial content of semen and there was no correlation between the total microbial counts, the proportion of these organisms sensitive to bovine seminal plasma inhibition and the measureable amount of antibacterial activity in a given semen sample. All semen samples had antibacterial activity, the amount being relatively constant for a given animal; marked differences occurred between breeds. Sixty-eight percent of the bacteria isolated from semen (representing ten genera) were inhibited by seminal plasma. Together with other data, this supports the view that most semen microorganisms are transient contaminants from outside the reproductive tract, derived mainly from the preputial cavity.

The most common organisms in bovine semen were species of Bacillus, Corynebacterium and Micrococcus, but twelve other bacterial genera and two yeast species were also represented among the isolates. Most semen microorganisms were sensitive to several antibiotics, with pseudomonads, coliforms and yeasts proving the most resistant. The control of these organisms is important since they may grow in extended semen and have been implicated in bovine genital disorders. Chloramphenicol and streptomycin are routinely used in semen extenders and have been highly effective in controlling bacterial growth; penicillin has been less effective.

At least three antimicrobial activities have been observed in bovine seminal plasma: (i) an antiviral activity, (ii) a heat labile (60°C for 60 minutes) antibacterial activity and (iii) a heat stable antibacterial activity. Research has centered on the heat stable fraction which is sensitive to proteolytic enzymes but not to periodate treatment. This heat stable fraction can be purified by adsorption onto highly polymerised deoxyribonucleic acid (DNA). It does not resemble lysozyme, polylysine, salmine or spermidine in antimicrobial spectrum, electrophoretic properties or amino acid composition. Data from dialysis, polyacrylamide gel electrophoresis, ultrafiltration and ultracentrifugation indicate that the active moiety occurs in varying molecular weight aggregates, caused by self association and interaction with other microsolute present in its environment. At least two of the aggregates (cationic at pH8.6) are antibacterial by the overlay technique which detects biological activity in electrophoresed proteins. However, it migrates as a single band (molecular

weight 25,000) in sodium dodecyl sulphate (SDS) polyacrylamide gel electrophoresis, and has a single N terminal amino acid. When compared with antibacterial compounds described by other workers, the antimicrobial proteins from bovine seminal plasma were found to have a unique chemical composition.

Bovine seminal plasma proteins had a rapid lethal effect on both Gram-positive and Gram-negative bacteria as well as ureaplasmas and mycoplasmas. Bacterial resistance to bovine seminal plasma exhibited a degree of correlation with resistance to antibiotics affecting cell membrane integrity. Detection of cytoplasmic leakage, loss of accumulated labelled compounds and failure to accumulate a labelled amino acid by bacteria in contact with seminal plasma supports the hypothesis that membrane damage is induced by bovine seminal plasma antimicrobial proteins. Resistance of Pseudomonas aeruginosa to bovine seminal plasma appears to be due to the bacterial cell wall preventing access of the active moiety to the cell membrane.

Bovine seminal plasma antimicrobial cationic proteins may well contribute to an efficient primary nonspecific defence mechanism of the genital tissues. Few microorganisms colonise the upper reproductive tract; ascending infections from the lower urethra and preputial cavity, where bacterial populations are large, are uncommon. Only bacteria shown to be resistant to antimicrobial factors present in bovine seminal plasma have been implicated in bovine male reproductive tract infections. During coitus the antimicrobial activity may also protect the female genital tract from infection.

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