THE FEMALE REPRODUCTIVE TRACT IMMUNE SYSTEM
VI ASSAY OF THE OPSONIC ACTIVITY OF THE FEMALE PIG GENITAL SECRETIONS

BY

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Introduction

Antimicrobial activity in secretions of the female genital tract of human (Rozansky **et al,** 1962) and domestic animals (Brownlie and Hibbit, 1972) has been recognized for a number of years. Some reports have referred to the presence of non-specific antimicrobial agents such as lysozyme, lactoferrin and peroxidase in secretions of the reproductive ductive tract of some species (Schumacher and Wied, 1967; Bushana **et al,** 1973; Linford, 1974) and a role was suggested for such agents in local defence (Klebanoff and Smith, 1970; Govers and Girard, 1972). The presence of complement components in these secretions however, have recieved little attention: only C_3 complement components were detected in human cervical mucus (Schumacher, 1971) and recently in rabbit fallopian tube fluid (Oliphant **et al,** 1977). Since complement is an essential mediator for immune bacteriolysis and features strongly in the immune mechanisms (Lachmann, 1978), the present study was undertaken to detect, indirectly, C_3 complement components in cervico-vaginal and uterine secretions of pigs by assaying the opsonic activity of the secretions using the alternative pathway mediated phagocytosis of yeast particles.

Material and Methods

1. Collection of genital tract secretions:

Cervico-vaginal secretions were collected from normally cycling sows by tampons following the methods of Szabo (1951). Briefly a tampon was pushed into the vaginal lumen as far as the cervical OS using a lubricated surgical speculum. The tampon was left in position for 1-2 h to absorb the secretions in the vaginal lumen at that site as well as secretions descending from the cervical canal. The tampon was then pulled out and placed in an empty 50 ml syringe. The absorbed secretions were extracted by compressing the tampon with a syringe plunger or by further moistening the tampon with 1-3 ml saline prior to compression.

Uterine secretions were collected from slaughtered animals. The uterine horns were removed immediately after slaughter and each horn was flushed with about 50 ml saline, gently massaging the uterine walls when the flushing fluid was inside. The flushing contents of both horns were pooled and then concentrated about 70-fold in carbowax (Gurr, London) at 4°.

All cervico-vaginal and uterine secretion samples were tested for the absence of blood with Hema-C ombistix (Ames, Miles Labs, England). Samples containing blood were discarded, negative samples were centrifuged at 18,000 g for 30 min. at 4°. The supernatants were separated and stored at -70° untill studied. The stage of the oestrous cycle of sampled animals was recorded.

2. Opsonization test:

(a) Principle:

The opsonizing activity of sow uterine and cervico-vaginal secretions was measured by counting the number of yeast particles phagocytosed by normal pig polymorphonuclear leucocytes when incubated in the presence of the test sample. The methods primarily adopted by Miller **et al** (1968) were applied.

(b) Method:

A suspension of Baker's yeast in saline was heated in a water-bath at 100 °for 30 min, filtered through gauze and resuspended in saline at 1×10^9 particles per ml. Normal polymorphs were separated from pig blood by dextran sedimentation. The leucocyte rich supernatant was washed three times and the polymorphs resuspended in saline at 5×10^6 cells per ml.

The test was performed in 2.5 ml stoppered plastic tubes. To O.1 ml of the test fluid, 0.2 ml of the leucocyte suspension and 0.1 ml yeast suspension were added. The tubes were shaken gently, incubated at 37 for 45 min followed by centrifugation at 50 g for 5 min. The supernatant was removed, and the deposit was resuspended in two drops of saline. A spread drop was dried on a slide and stained by the May- Grunwald/Giemsa method. At least 50 neutrophil polymorphonuclear leucocytes were identified in areas where they were close, but not clumped, and the number of yeast particles in each was recorded.

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The opsonization index, the mean number of yeast particles per polymorph, was determined. Serum samples from normal sows were run as a positive control with each test, and a saline negative control. The comparison of the opsonization indices of the different secretions and serum was performed parametrically using Student's t-test. p values $\angle 0.05$ were considered significant.

Results

The opsonic activity of sow reproductive tract secretions was measured in 6 cervico-vaginal secretions and 6 concentrated uterine washings obtained from sows at different stages of the oestrous cycle. The opsonization index (see fig. 1) for each sample and the mean $\pm$ SD for the whole group are shown in Table 1 and Fig 2. A mean background opsonization index of 1.55 $\pm$ 0.21 was recorded for the control saline samples. The opsonization indices of the serum and secretion samples were significantly higher than this value. The mean opsonization index of the uterine washings was 2.33 compared to 2.25 for the cervico- vaginal secretions, but the difference was not significant although the secretions were collected by different methods from the two sites of the tract and were not standardized to a certain concentration before applying the test. There was no apparent influence of hormones on the opsonic activity of the secretions, and the opsonization indices of the samples were nearly constant at different stages of the oestrous cycle. On the other hand, the mean opsonization index of normal sow serum was 3.93, as determined from 3 sows (Table 1, Fig 2). This value was higher than that of the uterine and cervico-vaginal secretions, with a highly significant difference ($p < 0.001$).

Discussion

Opsonization is a complex function, involving at least complement and/or antibody (Quie **et al,** 1968; Solberg et al, 1976). Since yeast and yeast extract zymosan activate complement directly through the alternative pathway (C3) (Gotze and Muller-Eberhard, 1971), it is likely that the yeast assay will not be dependent on the host antigenic experience (Soothill and Harvey, 1976). Opsonization of yeast particles by cervico-vaginal and uterine secretions of the pig as demonstrated by their significantly higher indices compared to the spontaneous background indices, is therfore an indication that components of the alternative complement pathway are present in these secretions. There was no significant difference between the opsonization potential of uterine and cervico-vaginal secretions and no evidence of hormonal influences was presented in the results, but it was apparent that alternative pathway mediated opsonization was more efficient in normal sera than in those secretions. This may be because the complement components were present in lower concentration in these secretions or diluted as pure secretions were not collected. Alternatively those secretions may lack some serum factor responsible for enhancing phagocytosis (Gigli **et al,** 1976). The presence of C_3 in the female genital secretions was reported in human cervical mucus (Schumacher, 1971) and there is evidence that it can be synthesised in small amounts by human vaginal tissue (Lai a fat **et al,** 1973), although it was not possible to localize C_3 or other complement components in the vaginal tissue with a fluorescein- tagged polyspecific anti-complement serum (Lai a Fat **et al,** 1974). The participation of C_3 in the local immunological activity of the female reproductive tract is possible since the alternative pathway can opsonize bacteria and mediate the bactericidal reaction (Frank **et al,** 1973). It seems likely that these C_3 components together with other elements present in the female genital secretions, such as lysozyme and immunoglobulins, may act synergistically to form an efficient immunological barrier against infection (Hussein, 1979). But however, presence of other complement components in these secretions, particularly the thermolabile C_{1q} which binds to immunoglobulin heavy chains and triggers the cascade of the classical complement pathway, remains to be confirmed.

TABLE 1
Opsonization Indices (Mean Yeasts/Polymorph) of Sow Serum and Reproductive Tract Secretions

Sample Number	Test Material	Hormonal State	No. of Polymorphs Counted	Opsonization Index	Mean Opsonization Index + SD
1	Conc. Uterine Washings	Lactating Sow	59	3.00	
2	"	Proestrus	52	2.20	
3	"	"	65	2.15	
4	"	Dioestrus	65	2.17	2.33 ± 0.33
5	"	"	58	2.30	
6	"	"	59	2.14	
1	Cervicovaginal secretion	Lactating Sow	61	2.80	
2	"	Proestrus	56	2.30	
3	"	"	59	2.20	
4	"	Oestrus	58	2.05	2.25 ± 0.29
5	"	Dioestrus	59	2.10	
6	"	"	60	2.05	
1	Normal Sow Serum	Proestrus	52	3.60	
2	"	Dioestrus	53	4.20	3.93 ± 0.31
3	"	"	52	4.00	
1	Saline Control		52	1.40	
2	"	—	53	1.70	1.55 ± 0.21

Summary

The opsonizing activity of swo uterine and cervico-vaginal secretions was measured by counting the number of yeast particles phagocytosed by normal pig polymorphonuclear leucocytes when incubated in the presence of the test material. The mean opsonization index was 2.33 and 2.25 for the uterine and the cervico-vaginal secretions respectively. The mean opsonization index of normal sow serum was 3.93 which is significantly higher than that recorded for the secretions. The results indicated that C_3 components are present in the female pig genital secretions and that may be of significance for protection against infection at the local level.

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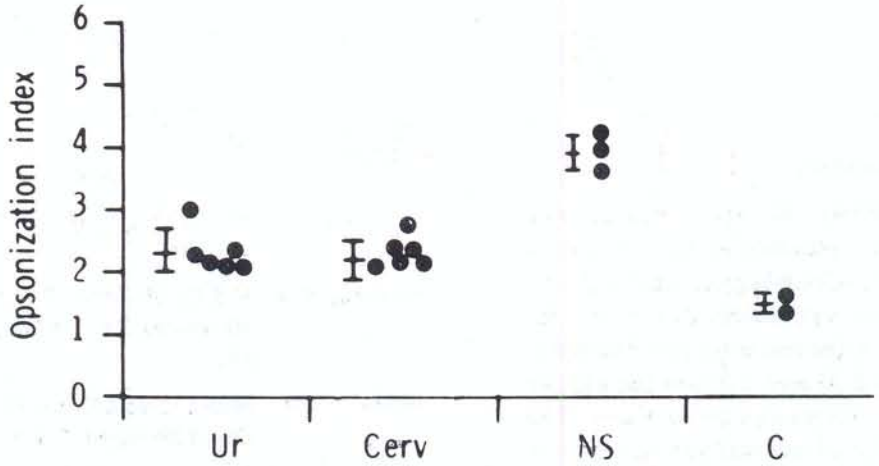
Fig 2 Opsonization indices of sow cervico-vaginal and uterine secretions. Points indicate samples; bar indicates mean ± S D.



Opsonization indices (mean yeast polymorph) of sow uterine (Ur) and cervico-vaginal (Cerv) secretions. Normal sow serum (NS) and saline (C) samples represent positive and negative controls respectively.

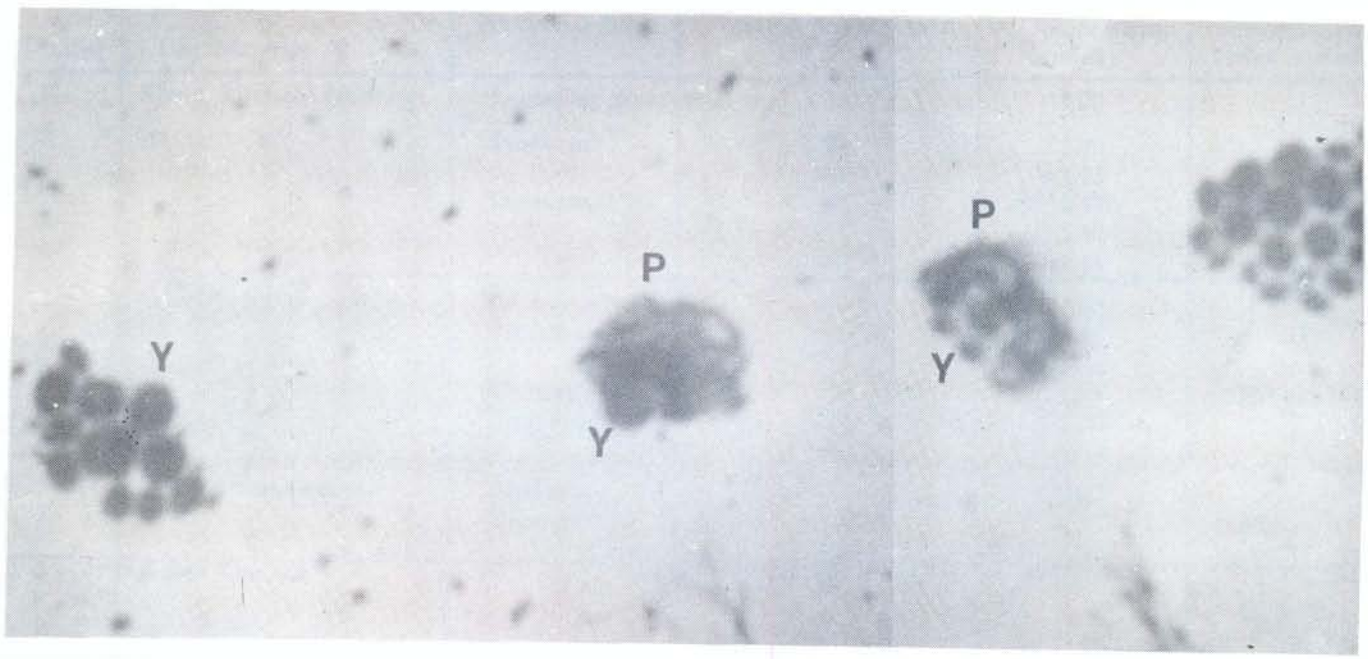


Fig (1)

Yeast particles (Y) Phagocytosed by normal pig Polymorphs (P) following opsnization by secretions from the sow genital tract. (Magnification X 1250).