THE FEMALE REPRODUCTIVE TRACT IMMUNE SYSTEM V. IMMUNOGLOBULIN CONTENTS OF PIG FALLOPIAN TUBE SECRETIONS

By

A.M. Hussein; F.J. Bourne * and T. Newby *

Veterinary Research Administration
P.O. Box. 8067—Alamarat
Khartoum—Sudan

Introduction

The electrophoretic pattern of mammalian fallopian tube fluid supports the view that the protein composition of the tubal fluid arises from both transudation and active secretion. The major serum proteins were found in the fallopian tube fluid in different concentrations to that in serum, in addition to the presence of specific tubal proteins (Moghissi, 1970; Shapiro **et al,** 1971; Roberts **et al,** 1976). Among these proteins immunoglobulins have been detected in the fallopian tube secretions of some animal species including the rhesus monkey (Marcus and Saravis, 1965), sheep (Roberts **et al,** 1976), rabbit (Shapiro **et al,** 1971) and the hamster (Johnson, 1973) by immunoelectrophoretic techniques or by electrophoresis on polyacrylamide gel. However, exact quantitation of immunoglobulins was carried out only in man and rabbit fallopian tube secretions (Lippes **et al,** 1972; Oliphant **et al,** 1977). The presence of immunoglobulins in follicular fluid and in other secretions bathing the female reproductive organs has been reported in man and animals (Chodirker and Tomasi, 1963; Edwards, 1968; Curtain **et al,** 1971), and the significance of these immunoglobulins in raising a local immune system capable of attacking invading pathogens has been established (Chipperfield and Evens, 1975; Corbeil **et al,** 1976). The purpose of this report is to give an account of the immunoglobulin classes and contents of pig fallopian tube secretions and to detect possible changes in the immunoglobulin ratios and concentrations in relation to influences of the ovarian hormones and other physiological variations.

* Veterinary School – Langford House – Langford, Bristol – England.

Materials and Methods

1. Collection of fallopian tube secretions:

Fallopian tube secretions were immediately collected from slaughtered animals by washing the tube with saline. The tube was first removed from the genitalia and clamped with artery forceps at the ovarian end (infundibulum) and the flushing material was introduced via the uterine end (isthmus) by a blunt end Pasteur pipette. Each tube was flushed twice with 1 ml saline. The washings of both tubes were pooled, checked for the absence of blood using a Hema-combistix (Ames, Miles Labs., England) and then centrifuged at 18,000 g for 30 min at 4°. The supernatant was concentrated approximately 15-20 times by ultrafiltration in a Minicon concentrator (Amicon, Ltd. U.K.) at 4° and stored at-18°.

2. **Preparation of antisera :**

Porcine immunoglobulins IgG, IgM and IgA were isolated from serum and milk as previously described (Bourne and Curtis, 1973). Antisera to these immunoglobulins were raised in sheep and rabbits by the methods of Bourne (1969) and rendered specific by solid phase immunoadsorption (Newby **etal,** 1974). Specificity of each antiserum was determined by immunoelectrophoresis and immunodiffusion.

3. **Quantitation of immunoglobulins :**

Quantitation of IgG, IgA and IgM in washings of the fallopian tube was performed by electroimmunoassay using specific antisera and serum standards. The modified methods of Weeke (1973) were used. Results are expressed as mg/ml.

4. **Statistical methods :**

A log transformation of the concentration of immunoglobulins was performed to normalize the data before calculating the mean, the standard deviation (SD) and the immunoglobulin concentrations ratio. The comparison of two immunoglobulin concentrations in a group of animals was performed on log transformed data using Student's t-test. A non-parametric procedure, Spearman's Correlation Coefficient-rs (Siegel, 1956), was used to investigate the correlation between two immunoglobulin concentrations in different samples. p values <0.05 were considered significant.

Results

Immunoglobulin levels and concentration ratios were studied in the fallopian tube washings of sows during the dioestrous (progesterone) and proestrous (oestrogen) phases of the cycle and in washings from prepubertal (not cycling) gilts (Table 1, Fig. 1). Sampling was avoided during oestrus and metoestrus due to blood contamination following follicular rupture and the passage of the ova down the tube.

IgG and IgA were detectable in low levels in the follopian tube concentrated washings; the highest mean immunoglobulin level was 0.26 mg/ml for IgG and 0.032 mg/ml for IgA while all samples studied were completely negative for IgM (Table 1). IgG was the predominant immunoglobulin and the difference was significant when compared to IgA concentration during dioestrus ($p < 0.025$), proestrus ($p < 0.01$) or in secretions of prepubertal gilts ($p < 0.001$). IgA was present in trace amounts in some samples from normally cycling sows and completely negative in the majority of samples of prepubertal gilts. The mean IgG: IgA ratio was 8.12:1 during dioestrus and dropped to 6.15:1 at proestrus, but the difference between ratios at both phases of the cycle was not significant (Table 1, Fig 1). IgG formed the bulk of immunoglobulin in fallopian tube secretions of prepubertal gilts and the mean IgG: IgA ratio was not estimated as IgA could not be detected in the majority of samples.

The correlation between IgG and IgA concentrations in the fallopian tube washings was just significant ($p = 0.05$) in dioestrous secretions (Table 2) while there was no correlation between the two immunoglobulins in proestrous secretions.

Discussion

The quantitative data of this study indicated a low immunoglobulin content in pig fallopian tube fluid which is consistent with the depressed immunoglobulin level, compared to serum, normally found in the tubal fluid of the rhesus monkey (Marcus and Saravis, 1965), rabbit (Oliphant **et al,** 1977) and man (Lippes **et al,** 1972) although a boost of immunoglobulin level in the tubal fluid may occur at ovulation following the release of follicular fluid, which is rich in immunoglobulins (Johnson, 1973).

IgG is the predominant immunoglobulin in pig fallopian tube secretions followed by IgA while IgM could not be detected. Low level or absence of IgM in the tubal fluid was also reported in man (Moghissi, 1970; Lippes **et al,** 1972) and in the rabbit (Oliphant **et al,** 1977). The paucity of IgM in the fallopian tube secretions and in other secretions bathing the female reproductive tract (Duncan **et al,** 1972; Menge and Lieberman, 1974; Chipperfield and Evans, 1975; Corbeil et al, 1976) may indicate that the concentration of immunoglobulin in these secretions is controlled, in part, by selective serum transudation based on molecular size (Chandra **et al,** 1974; Whitmore and Archbald, 1977) and IgM being a large molecule is therefore more restricted to the intravascular space (Waldman, 1969; Wilkie **et al,** 1972).

The IgG: IgA ratio 6.15:1 and 8.12:1 of sow tubal fluid was slightly less than the corresponding ratio of 10.2:1 reported for sow serum (Bourne, 1971). This suggests a small amount of IgA synthesis by sow fallopian tube tissue, particularly considering that IgA levels were probably underestimated in this study by using 7S serum IgA standards for quantitating secretory IgA. A predominance of IgG was also reported in human tubal fluid by Lippes **et al** (1972) although their results indicated no IgA synthesis as the IgG: IgA ratio resembled that of serum. However, local synthesis of IgA by the rabbit fallopian tube was suggested following the determination of an IgG : IgA ratio of 1:1.38 in the tubal fluid compared to a corresponding ratio of 50:1 in serum (Lieberman and Menge, 1971; Oliphant et al, 1977), while in the cow no immunoglobulin could be detected in the fallopian tube flushings, even after concentration (Corbeil **et al,** 1976). The different methods used for collecting the secretions in the above studies and perhaps species variations accounts for these contradictory findings in the immunochemical analysis. Moreover, serveral questions may be raised in attempting to define a local immunoglobulin synthesis by either immunoglobulin quantities or ratios without considering presence of immunoglobulin synthesising cells at the site, selective transport of immunoglobulins across mucous membranes into the lumen and half lives of immunoglobulins in the secretions.

The hormonal influences on the immunoglobulin ratio and concentrations in the porcine fallopian tube secretions was not significant. This agrees with the findings in the tubal fluid of man (Lippes et al 1972) and the rabbit (Oliphant et al, 1977) although in the latter IgM was detected only in oestrus fallopian tube

secretions. However, in the present study the bulk of tubal fluid immunoglobulin of prepubertal animals was IgG, IgA being undetectable in the majority of samples. This low level of IgA may be associated with the lack of antigenic stimulation at the fallopian tube mucous surface of these maiden animals, such stimuiation being necessary for the development of IgA in secretions (Mattioli and Tomasi, 1973; Tomasi, 1976).

The weakly positive correlation between the IgG and IgA concentrations in pig fallopian tube secretions during the dioestrous phase of the cycle suggests the similarity of factors governing the presence of both immunoglobulins, whether local synthesis (Mattioli and Tomasi, 1973; Tomasi, 1973).

The wealy positive correlation between the IgG and IgA concentrations in pig fallopian tube secretions during the dioestrous phase of the cycle suggests the similarity of factors governing the presence of both immunoglobulins, whether local synthesis (Oliphant **et al,** 1977) or serum transudation (Lippes **et al,** 1972), while the lack of correlation during the proestrous phase may suggest either a loss of selective serum/mucus transudation or more locally synthesised IgA, which perhaps resulted in the insignificant decrease in the IgG:IgA ratio compared to that of dioestrous secretions.

The findings presented in this study indicated that mammalian fallopian tube secretions contain immunoglobulins; these immunoglobulins may play a functional immunological role as they may constitute antibodies to protect the mucosa or the ova as they pass down the tube (Johnson, 1973; Whitmore and Archbald, 1977). But, however, immunological implications related to infertility may arise if these immunoglobulins constitute antibodies directed against spermatozoal antigens (Menge, 1970; Edwards, 1974).

Summary

IgG, IgA and IgM were quantitated in the fallopian tube washings of pigs by electroimmunoassay, using specific antisera and serum standards. The washings were concentrated 15-20 times prior to quantitation. The highest mean immunoglobulin levels was 0.26 mg/ml for IgG and 0.032 mg/ml for IgA while all samples studied were completely negative for IgM. There was no significant difference in immunoglobulin concentrations and ratios between samples taken during the dioestrous and the proestrous phases of the cycle. However, there was a weak positive correlation between IgG and IgA concentrations in dioestrous samples only. The bulk of tubal fluid immunoglobulins of prepubertal animals was IgG, IgA being undetectable in the majority of samples. The IgG: IgA ratio of sow fallopian tube secretions was slightly less than that recorded for sow serum, which probably indicates a small amount of IgA synthesis by sow fallopian tube tissue.

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Fig 1 IgG:IgA ratios of concentrated washings of pig fallopian tube at dioestrus and proestrus phases of the cycle. Points represent samples. Bar indicates mean ratio for each group.

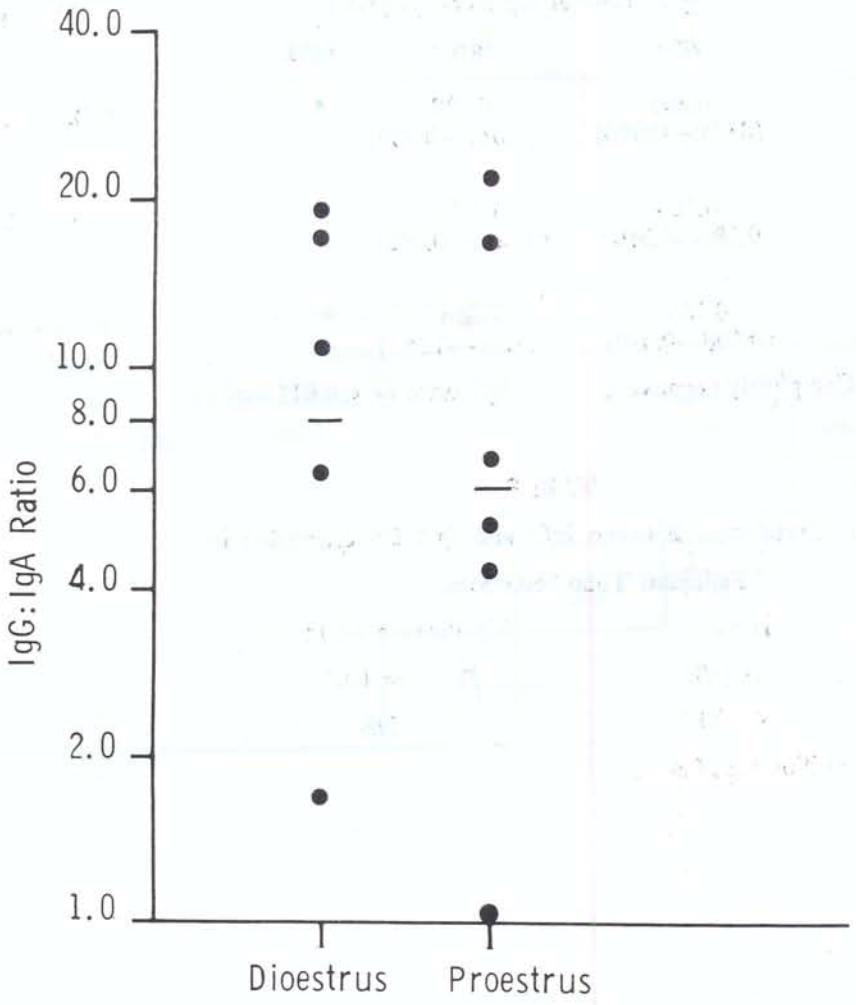

Table 1

Immunoglobulin Mean Concentration and Immunoglobulin Ratios in Pig Fallopian Tube Washings at Various Hormonal Stages

Hormonal Stage	No. in Group	Antilog of log mean concentration ± one SD of log mean (mg/ml) IgG	IgA	IgM	Immunoglobulin ratio IgG : IgA : IgM
Progesterone Phase (Dioestrus)	5	0.260 (0.070—0.940)	0.032 (0.011—0.080)	*	8.12 : 1 : —
Oestrogen Phase (Proestrus)	6	0.160 (0.084—0.310)	0.026 (Trace—0.080)	*	6.15 : 1 : —
Prepubertal (Not cycling)	9	0.185 (0.106—0.320)	Trace (Trace—0.03)	*	18.5 : Trace : —

* Completely negative . IgA trace = <0.011 mg/ml .

TABLE 2

The Correlation Between IgG and IgA Concentration in Fallopian Tube Secretions

Physiological State	n	rs value	Significance of rs value
Dioestrus	5	+ 0.90	p = 0.05
Proestrus	6	+ 0.17	NS

NS = Not Significant.