A STUDY OF UTERINE AND SERUM PROTEINS IN CASES OF FEMALE INFERTILITY OF UNKNOWN AETIOLOGY

by
V. K. SINGH,* M.S.
and
K. VISHNOL,**

Introduction

The uterine environment doubtless provides shelter and nourishment for the developing and implanting conceptus. During the whole period of gestation the embryo depends upon the milieu provided by the maternal organism and during the pre-implantation, there exists a well documented dependency of the blastocyst upon the maternal substrates provided by the uterine tissues. Uterine secretions change continuously in volume, viscosity and concentrations of their constituents (Kulangaxa, 1972). Shirai *et al* (1972) described a specific protein in the uterine fluid which plays an important role in the implantation of the conceptus. Wolf and Mastrionni (1975) have shown the presence of a low molecular weight protein in the uterine milieu which is responsible for the implantation.

Though various aspects of sterility have been widely studied, yet in some women we are not able to label its cause. This work has been primarily done to study the various proteins in the uterine fluid and to find their role in these infertile women where no other cause for their infertility is detected e.g. any defect in the male partner, absence of ovulation, blocked fallopian tubes, any other local factor hindering the implantation of ovum or disease in the mother attributable as the cause of sterility.

Material and Methods

Three hundred cases of sterility were selected from the out patient department of UISE Maternity Hospital and other nursing homes of Kanpur over a period of 2 years. Forty control cases were also studied. A detailed history and clinical examination were done and the relevant investigations e.g. endometrial biopsy, tubal insufflation test hysterosalpingography and semen examination of the husband were done in all the cases. The cases in whom we could find any cause of sterility were selected for this study. Uterine fluid and serum proteins were analysed in 150 cases only. Clear and careful instructions were given to these patients to come in the various phases of menstrual cycle viz. proliferative phase (5th day of menstrual cycle), early secretory phase (16th to 21st day) and late secretory phase (22nd to 28th day) for collection of samples and follow up.

Six ml of normal saline was injected into the uterine cavity by means of a 2 mm bore metal cannula, out of which 3-4 ml of uterine washing could be collected through the same cannula and kept in a plain vial. Samples were centrifuged immediately for removal of any particular

*Reader in Obstet. & Gynec.
**Research Assistant in Pathology.
G.S.V.M. Medical College, Kanpur.
Accepted for publication on 25-3-1980.

matter and kept at 4°C to be analysed. Simultaneously 5 ml of blood was also collected from every patient in plain sterile tubes, centrifuged and serum kept for analysis. The uterine fluid washing and blood samples were studied in all the three phases of menstrual cycle in all the patients.

Protein content estimation of the uterine washings was done by Hartree's modification (1972) of Lowry *et al* method (1951) that gives a linear photometric response. Polyacrylamide-gel electrophoresis was employed to analyse the different types of proteins in serum and uterine fluid washings.

Observation

Out of 150 cases, the incidence of primary sterility was higher 60.66% (91 cases) than that of secondary sterility which was 39.34% (59 cases). The average age of the primary sterility cases was 25 to 30 years, and that of secondary sterility cases was slightly higher 28 years, although their commonest age group was the same i.e. 25 to 30 years.

Area wise distribution (rural/urban), caste and religion had no bearing in the present study.

Besides the complaints of infertility the commonest complaint in both primary as well as secondary sterility group was pain in abdomen (42.00% & 81.30% respectively), white vaginal discharge (67.40% & 62.50% respectively) and menstrual disorders (44.00% & 21.80% respectively). The menstrual pattern was normal in 55.30% of primary and 48.30% of secondary sterility. The most common menstrual disorder in primary sterility was scanty menstruation found in 36.40% cases but in secondary sterility dysmenorrhoea and menorrhagia were the commonest complaints (46.70% & 34.60% respectively).

Table I shows the total protein values in uterine fluid of the control cases.

The highest total protein content i.e. 2.01-2.50 mg/100 ml was found in 1 case (8.1%) in the late secretory phase.

Table II shows the total protein values in uterine fluid in cases of primary sterility.

The range of protein values in various phases was observed to be 0.11-2.00 mg in the proliferative phase, 0.11-1.50 mg in the early secretory phase and 0.11-2.00 mg/100 ml in the late secretory phase.

Table III shows the protein values in

TABLE 1

Total Protein Value in Uterine Fluid

Sl. No.	Protein values mg/100 ml	Proliferative No.	%	Early secretory No.	%	Late secretory No.	%
1.	0.00-0.05	—	—	—	—	—	—
2.	0.06-0.10	—	—	—	—	—	—
3.	0.11-0.50	2	13.3	6	50.0	—	—
4.	0.51-1.00	5	33.3	4	33.3	4	30.8
5.	1.01-1.50	6	40.0	2	16.7	8	61.6
6.	1.51-2.00	2	13.4	—	—	—	—
7.	2.01-2.50	—	—	—	—	—	—
8.	2.51-3.00	—	—	—	—	—	8.1
	Total	15		12		13	
	Control cases 40	Range 0.39-1.80		Range 0.16-0.01		Range 0.68-2.48	

TABLE II

Total Protein Values in Uterine Fluid in Primary Sterility

Sl. No.	Protein values mg/100 ml	Proliferative No.	%	Early secretory No.	%	Late secretory No.	%
1.	0.00-0.05	—	—	—	—	—	—
2.	0.06-0.10	—	—	—	—	—	—
3.	0.11-0.50	3	17.7	15	41.5	6	24.0
4.	0.51-1.00	15	48.4	13	36.1	12	48.0
5.	1.01-1.50	10	32.2	8	22.4	5	20.0
6.	1.51-2.00	3	17.7	—	—	2	8.0
7.	2.01-2.50	—	—	—	—	—	—
	Total	31		36		25	

TABLE III

Total Protein Value in Uterine Fluid in Secondary Sterility

Sl. No.	Protein values mg/100 ml.	Proliferative No.	%	Early secretory No.	%	Late secretory No.	%
1.	0.00-0.05	—	—	—	—	—	—
2.	0.06-0.00	—	—	—	—	—	—
3.	0.11-0.50	8	34.7	11	64.7	2	11.1
4.	0.51-1.00	10	43.6	6	35.3	9	50.0
5.	1.01-1.50	—	—	—	—	5	27.0
6.	1.51-2.00	5	21.7	—	—	2	11.2
7.	2.01-2.50	—	—	—	—	—	—
	Total	23		17		18	
	Range	0.11-2.00		0.11-2.00		0.11-2.00	

uterine fluid in secondary sterility group.

Table IV shows the average total protein content in uterine fluid during different phases of menstrual cycle in control, primary and secondary sterility cases.

The protein values were lower in the primary and secondary sterility cases in all the phases of menstrual cycle than in the control cases.

Paper Electrophoretic Study: At least 5 bands were obtained in all the serum samples of the control cases. The same 5 bands were observed in the serum of primary and secondary sterility cases. In fact no difference in the bands in the serums of control, primary and secondary sterility cases was recorded. In the uterine flushing samples 28% cases

TABLE IV

Protein Content in Uterine Fluid in Different Phases of Menstrual Cycle

Sl. No.	Type of case	Protein values in mg/100 ml		
		Proliferative	Early secretory	Late secretory
1.	Control	1.04	0.48	1.10
2.	Primary sterility	1.19	0.46	0.70
3.	Secondary sterility	0.58	0.41	0.88

showed a ß-globulin band in the late secretory phase, 34°% showed an albumin band in the proliferative phase, 28% cases showed this band in the early secretory phase and 25% cases showed this band in the late secretory phase.

In the sterility group band of albumin was seen in all cases of primary as well as secondary sterility, but ß-globulin band was present in only 16 cases out of (40%) 40 cases of primary sterility and 33 out of 54 (61.1%) cases of secondary sterility in whom electrophoretic studies were done.

Thus paper electrophoresis revealed different patterns of protein bands in both serum and uterine flushings. The bands seen are those of albumin, alpha-1, alpha-2, beta 1, beta 2 and gamma globulins.

Table V shows protein band paterns in normal control cases during paper electrophoresis.

As seen in the Table V 66.6% cases showed band in albumin region in proliferative phase and 83.3% showed this band in the same region in early secretory phase, while 60% showed a band in the albumin region in the late secretory phase. A band in the ß-globulin region was seen in 33.4% cases in proliferative phase, 16.7% cases and 40.0% cases in early and late secretory phases respectively. Protein band pattern in the uterine fluid of primary and secondary sterility cases was not definite. Only Albumin bands were seen in all the three phases. Globulin bands were observed only in a few cases of each group.

Gel Electrophoresis: Gel Electrophoresis is a more sensitive technique as compared to paper electrophoresis and by this method we could observe about nine bands of protein in the four well defined zones, namely albumin; pre-albumin; transferrin and post transferrin. The broadest and farthest moving zone was post transferrin. It showed a minimum of 6 bands in 96% cases.

The relative mobilities of serum protein bands and bands of uterine flushing was calculated with respect to albumin in all the three groups of patients i.e. control, primary sterility and secondary sterility in all the phases of menstrual gycle.

$$Rm = \frac{\text{Mobility of any protein band}}{\text{Mobility of serum albumin}}$$

We found that all the protein bands with relative mobility ranging from 0.39 to 0.86 show an incidence of more than 72.72% in the control group in about 80% cases in primary sterility group. In secondary sterility cases, band with Rm 0.71 was present in the early secretory

TABLE V

Protein Band Patterns in Normal Control Cases

Sl. No	Region of Protein band	Proliferative phase		Early secretory phase		Late secretory phase	
		No.	%	No.	%	No.	%
1.	Albumin	16	66.6	5	83.3	6	60.0
2.	B Globulin	8	33.4	1	16.7	4	40.0
3.	Other bands	—	—	—	—	—	—

phase in 72% cases. Paper electrophoretic and gel electrophoretic findings could not be compared with others as no other reports were available on this subject.

Discussion

In the present series qualitative as well as quantitative estimation of serum proteins of uterine flushing was done in 40 controls, 92 cases of primary sterility and 58 cases of secondary sterility. In all these cases no cause of sterility could be identified.

In control cases our observations regarding the variation of total protein content in the uterine flushing are in agreement with Robert *et al* (1976) who have also shown an elevated protein content in the late secretory phase (Specially just before menstruation) and also in the early stage of proliferative phase, remaining relatively constant during the rest of the menstrual cycle. Murray *et al* (1972) have also made similar observation. In the present study the maximum protein content in proliferative phase was 1.80 gm/100 ml, in early secretory 1.01 gms/100 ml in late secretory 2.48 gm/100 ml, thus we see that there is a definite elevation in the protein content in the proliferative and late secretory phase although this difference is not very significant owing to large variation in each group.

The protein content of uterine flushing, in the present study was lower than the value reported by the other workers like Daniel (1971) and Robert *et al* (1976). Their lower values are probably due to the fact that the total plasma protein content of our cases is lower than the average protein content reported from other countries and since the uterine secretions are derived from blood, the protein content of uterine fluid would reflect the serum protein content of the patient.

In primary sterility subjects we found that the protein content was highest in the late secretory phase and lower in the early secretory phase. We could not compare this finding of ours with others because no reports were available.

In the secondary sterility cases the protein values were lower as compared to the primary sterility and control cases, the lowest content being present in the proliferative phase i.e. 0.58 gm/100 ml. as compared to the values of 1.19 gm/100 ml in the primary sterility, and 1.04 gm/100 ml in the normal control group. These results again could not be compared with others as no work has been published on this subject to the best of our knowledge.

Summay and Conclusions

1. Total protein content of uterine flushing of normal control cases, primary and secondary sterility group was in the range of 0.12-2.4 mg/100 ml.

2. The total protein content of uterine flushing in secondary sterility cases showed a definite decline during proliferative early and late secretory phase.

3. The total protein content in the primary sterility cases was lower on the early and late secretory phase but was higher in the proliferative phase as compared to control cases.

4. Paper electrophoresis of serum did not show any difference in the control, primary and secondary sterility cases.

5. The gel electrophoresis of serum of control, primary and secondary sterility cases showed no difference. In 94% cases 9 bands were seen.

Only 3-6 bands were seen in the gel electrophoresis in uterine flushing of normal as well as primary and secondary sterility cases.

Seven bands were seen in the proliferative phase of normal control cases, 5 bands in the same phase of primary sterility cases and 6 bands in the secondary sterility cases. In the early secretory phase 8 bands were seen in normal cases, 7 bands in primary sterility cases, 5 bands were seen in secondary sterility cases.

References

1. Daniel, J. C. Jr.: Adv. Biosc. 6: 191, 1971.
2. Hartree, E. P.: Analytical Bio. Chem. 23: 929, 1972.
3. Kulangaxa, A. C.: J. Reprod. Fert. 28: 419, 1972.
4. Lowry, O. H., Rose Brough, M. J.: Fan, A. L., Randall R. J.: J. Biol Chemistry 193: 265, 1951.
5. Murray, F. A., Bazer, Wallace, H. D., Warnick, A. C.: J. Bio. Reprod. 7: 314, 1972.
6. Robert, G. P., Parker, J. M., Henderson, S. R.: J. Reprod. Fertil. 48: 153, 1976.
7. Shirai, E., Lizuka, R. and Notake, Y.: Fertil. Steril 23: 522, 1972.
8. Wolf Don, P., Mastrionni, L. Jr. Fertil. Steril. 26: 240, 1975.