Results of SDS Electrophoresis and Immunoblotting

Electrophoresis

A- SDS electrophoresis revealed that several similar protein bands were present in the peritoneal fluid of all the three study groups.

B- There was a protein band corresponding to 32 (kd), molecular weight

1- present in all cases of endometriosis

2- present in 5/ 30 cases of unexplained infertility

3- absent in fertile control group

C- PF of endometriosis group contained higher number of protein bands (mean: 11, range = 10 to 22) compared to unexplained group (mean : 8 , range = 5 to 11) and control group (mean: 7 , range = 5 to 8).

Immunoblotting

A) Staining of the nitrocellulose by non-specific colloidal gold revealed the same results of SDS electrophoresis.

B)By alkaline phosphatase immun-blot kit. The cytokine TNF-α band appeared in the endometriotic and unexplained groups with different densities and appeared in 7 cases of the control groups.

Results of the cytokine TNF-α by ELISA

Table (3) shows the range and mean ± SD of the cytokine TNF-α levels in the 3 groups.

1. The cytokine level of TNF-α in the peritoneal fluid of both endometriosis (295.33 ± 63.41 pg / ml) and unexplained infertility groups (137.83 ± 39.811 pg / ml) were significantly higher than that in the control group(106.97 ± 20.721 pg / ml) ($P < 0.0001$)

2. The level of the TNF-α in the endometriosis group was statistically higher than that in the unexplained infertility group ($P < 0.0001$)

3. The mean intra-assay coefficient of variation was 2.7 %.

4. The mean inter – assay coefficient of variation was 5.0%

Table (3) :The level of peritoneal fluid cytokine TNF-α by ELISA in the 3 groups.

Group / TNFα level pg/ml	Control	Endometriosis	Unexplained infertility
Range	80-140	200-400	115-210
Mean	106.97	295.33	137.83
SD ±	20.721	63.461	39.811
P	-	3.814	15.618
T	-	< 0.0001	<0.0001

This table shows that the difference in the TNF-α level in endometriotic and unexplained groups were extremely higher than the control group (t ·· 0.0001)

Results of embryotoxicity of the peritoneal fluid in the 3 groups

The percentage of 2 cell mouse embryos that progressed to blastocysts in the peritoneal fluid supplements at 72 hours is shown in table 4.

1-Embryotoxicity of the peritoneal fluid of the endometriotic group was extremely significantly higher (there was only 57 embryos reached to the blastocyst stage out of 306 embryos cultured with the presence of PF of endometriotic group) compared to the control group (There was 280 embryos reached to the blastocyst stage out of 300 embryos cultured with the presence of PF of the control group) ($P < 0.001$)

2- Embryotoxicity of the peritoneal fluid of the unexplained infertility group was significiantly higher (there was 220 embryos reached to the blastocyst stage out of 310 embryos cultured with the presence of PF of unexplained in fertility group) than the control group ($P < 0.05$)

3- Embryotoxicity of the peritoneal fluid of the endometriotic group was significiantly higher than the unexplained infertility group ($P < 0.05$)

Table (4): Embryotoxicity assay of the peritoneal fluid in the 3 groups

Groups / Data	Control	Endometriotic	Unexplained Infertility
▪ **Number of blastocysts**	280	57	220
Total number of embryos	300	306	310
%	93.33%	18.62%	70.96%
Z		8.89	2.36
P		<0.001	< 0.05

The differences between the studied groups (endometriosis and unexplained infertility) and the control group were significiantly high (P 0.05)