

JULY 3, 1985

THE LANCET, AUGUST 3, 1985

233

HUMAN T-LYMPHOTROPIC VIRUS TYPE III (HTLV-III) INFECTION IN SERONEGATIVE HAEMOPHILIACS AFTER TRANSFUSION OF FACTOR VIII

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Summary Fifteen haemophilic patients acquired antibodies to human T-lymphotropic virus type III during 1984. One batch of factor VIII concentrate given to all these patients is presumed to be the cause of the seroconversion. A further eighteen patients who received the same batch did not seroconvert and one other patient became seropositive but had not received this batch. Before transfusion of the implicated batch the patients had low T-helper-cell numbers and T-helper/suppressor ratios; neither changed in those who seroconverted. The probability of seroconversion was independently related to the pre-existing low T-helper/suppressor ratio, the number of vials of the implicated batch transfused, and the total annual factor VIII consumption. Ten other patients received a batch of factor IX concentrate from the same donor plasma; none of these patients seroconverted.

Introduction

SUBSTANTIAL evidence has accumulated that the most likely cause of the acquired immunodeficiency syndrome (AIDS) is human T-lymphotropic virus type III (HTLV-III, otherwise known as lymphadenopathy-associated virus). Transfusion of red cells, platelets, or factor VIII and IX concentrates may result in the appearance of anti-HTLV-III, and in a small proportion of seropositive recipients symptoms of the AIDS-related complex or AIDS will develop.^{1,2} Tests for anti-HTLV-III on stored serum samples from haemophiliacs have shown that seroconversion was first detectable in 1978 in the United States,³ and no later than the following year in the United Kingdom (R. A. W., R. S. T., unpublished).

In contrast to haemophiliacs elsewhere in the UK, almost all patients attending the Edinburgh Haemophilia Centre have received factor VIII and IX concentrates prepared exclusively from locally collected plasma by the Scottish National Blood Transfusion Service (SNBTS). Until recently there were no reported cases of AIDS in Scotland and it therefore seemed possible that our patients might not have been exposed to HTLV-III. However, the long incubation period between infection and the appearance of AIDS means that some symptom-free carriers of HTLV-III might have donated blood. We have previously reported reductions in the absolute T-helper-cell numbers and the helper/suppressor ratio in our patients; because our donor population was apparently AIDS-free, we concluded that these immune changes were the result of infusion of factor VIII concentrates per se rather than infection by HTLV-III.⁴ We have now confirmed, by testing stored serum samples, that at the time of our previous study⁴ (spring 1983) all the patients who received solely SNBTS blood products did not have anti-HTLV-III.

As part of the continuing assessment of our haemophiliacs, we have now observed that sixteen of our patients acquired anti-HTLV-III during 1984; all but one of these patients had received a common batch of SNBTS factor VIII concentrate.

Patients and Methods

Anti-HTLV-III was detected as described previously⁵ in serum samples which had been collected periodically from all patients and stored at -20°C. Lymphocytes were counted with a 'Coulter S Plus'. Subsets were quantified by indirect immunofluorescence with T4 or T8 specific monoclonal antibodies in the first layer.⁶ Statistical analysis of differences between groups for any variable was done by the Wilcoxon rank sum test and of the changes within groups by the Wilcoxon signed rank sum test. The effect of several variables simultaneously on the probability of seroconversion was investigated by means of multiple linear logistic regression.

Thirty-four patients with haemophilia A (twenty-nine severe, five moderate) of mean age 27.6 years (range 10-49), eight patients with haemophilia B (four severe, four moderate) of mean age 26.2 years (range 8-56), and one patient with severe von Willebrand's disease (aged 56) were studied. Antibodies to factor VIIIc were present in three patients with severe haemophilia A. Serum samples collected in late 1983 and early 1984 were all negative for anti-HTLV-III.

During 1984 all patients in this study were treated on demand with multiple batches of SNBTS factor VIII concentrate of intermediate purity. In addition to factor VIII, two patients with haemophilia A with antibodies to factor VIIIc and all the patients with haemophilia B received SNBTS factor IX concentrate ('Defix'). The former two patients also received 'FEIBA' (Immuno). Two haemophilia A patients (one with an anti-factor VIII inhibitor) also received commercial high-purity factor VIII concentrates.

No patient was known to have risk factors for developing antibodies to HTLV-III other than the replacement therapy.

G. H. ELDER AND OTHERS: REFERENCES—continued

- de Verneuil H, Besumont C, Deyboch J-C, Nordmann Y, Sfar Z, Kastaly R. Enzymatic and immunological studies of uroporphyrinogen decarboxylase in familial porphyria cutanea tarda and hepatoerythropoietic porphyria. *Am J Hum Genet* 1984; 36: 613-22.
- Grossman ME, Bickers DR, Fob-Fitzpatrick MB, De Leo VA, Herbert LC. Porphyria cutanea tarda: Clinical features and laboratory findings in 40 patients. *Am J Med* 1979; 67: 277-86.
- Landrall O, Weisfeld A, Landin P. Iron storage in porphyria cutanea tarda. *Acta Med Scand* 1976; 188: 37-53.
- Ramsay CA, Magnus IA, Turnbull A, Baker H. The treatment of porphyria cutanea tarda by venesection. *Quart J Med* 1974; 43: 1-24.
- Kushner JP. The enzymatic defect in porphyria cutanea tarda. *NEngl J Med* 1982; 306: 799-800.
- Elder GH, Wyrill PC. Measurement of uroporphyrinogen decarboxylase using porphyrinogens prepared by chemical reduction. *Enzyme* 1982; 24: 186-95.
- Elder GH, Tovey JA, Sheppard DM. Purification of uroporphyrinogen decarboxylase from human erythrocytes. *Biochem J* 1983; 213: 45-53.
- Elder GH, Urquhart AJ. Human uroporphyrinogen decarboxylase. Do tissue-specific isoenzymes exist? *Trans Biochem Soc* 1984; 12: 661-62.
- De Salamanca RE, Pena ML, Chinarro S, et al. Quantitative and qualitative porphyrin excretion in normal subjects. *Int J Biochem* 1982; 14: 251-54.
- Smith AG, Francis JE. Investigations of rat liver uroporphyrinogen decarboxylase: Comparisons of porphyrinogens I and III as substrates and the inhibition by porphyrins. *Biochem J* 1981; 195: 241-50.
- de Verneuil H, Sassa S, Kappas A. Purification and properties of uroporphyrinogen decarboxylase from human erythrocytes. *J Biol Chem* 1983; 258: 2454-60.
- Halliwel B, Gutteridge JMC. Oxygen toxicity, oxygen radicals, transition metals and disease. *Biochem J* 1984; 219: 1-14.
- Elder GH, Sheppard DM. Immunoreactive uroporphyrinogen decarboxylase is unchanged in porphyria caused by TCDD and hexachlorobenzene. *Biochem Biophys Res Comm* 1982; 109: 113-20.
- Straka JG, Stahl DC, Kushner JP. Isolation and characterization of human hepatic uroporphyrinogen decarboxylase. *Clin Res* 1983; 31: 35A.
- de Verneuil H, Grankamp B, Foubert C, et al. Assignment of the gene for uroporphyrinogen decarboxylase to human chromosome 1 by somatic cell hybridization and specific enzyme immunoassay. *Hum Genet* 1984; 66: 202-05.

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Results

Between April and October, 1984, anti-HTLV-III developed in sixteen patients with haemophilia A (see table). The transfusion records of these patients showed that all but one had received a common batch (A) of SNBTS factor VIII between March and May, 1984. Of all the other batches of factor VIII transfused during this period, the next most likely implicated batch (B) was transfused during January, 1984, and was given to only nine of the sixteen patients who seroconverted. The source of HTLV-III in the one patient with severe haemophilia A who did not receive batch A remains obscure, but he did receive batch B. He had an orthopaedic operation in November, 1983, covered by SNBTS factor VIII, but since January, 1984, he had received treatment on only 3 occasions with factor VIII from batch B. He had not received any commercial factor VIII.

In addition to the fifteen patients who are known to have seroconverted a further eighteen patients received the implicated batch A. Serum samples from these patients have been collected at least 4 months (in some cases up to 8 months) after transfusion of batch A and have been negative for anti-HTLV-III. The shortest time from first infusion to the presence of detectable antibody was 31 days.⁶ However, for most patients it is difficult to estimate the minimum time between infusion and the development of the implicated batch (fig 1) and the serum samples were taken only periodically, mostly every few months. One of the patients who received 81 bottles of the implicated batch was negative at 20 weeks but positive at 40 weeks.

Lymphocyte subsets were investigated in twenty-four of the patients during the spring of 1983⁴ and in the autumn of 1984 (table). In 1983 the patients in whom anti-HTLV-III later developed had lower T-helper/suppressor ratios (mean 1.51, median 1.24) than those who did not seroconvert (2.11, 2.10) and the controls (2.05, 1.93). The difference between the two patient groups just failed to reach statistical significance (p=0.06). In 1983 the absolute T-helper-cell numbers in those who subsequently seroconverted were significantly lower than those in the controls, whereas there was no difference between the controls and those who did not seroconvert (table). T-suppressor-cell numbers were normal in both groups. There were no significant changes in helper and suppressor numbers or in the helper/suppressor ratio between 1983 and 1984.

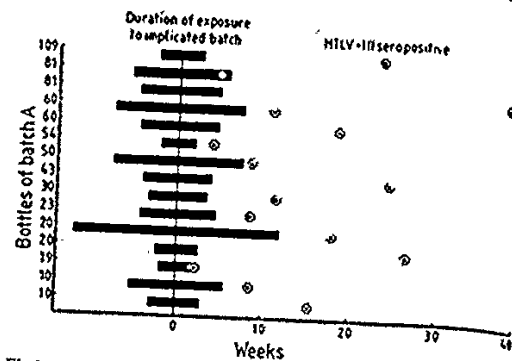


Fig 1—Relation between time of infusion of batch A, number of vials transfused, and development of anti-HTLV-III.

The fifteen patients who seroconverted used significantly more vials of batch A (p<0.01) and also had a higher annual factor VIII consumption (p<0.01) than the eighteen patients who did not seroconvert (figs 2 and 3). These three factors were assessed simultaneously by applying multiple linear logistic regression analysis to the data from the 24 patients with complete observations. The effect of the helper/suppressor ratio was significant (p<0.02); the number of vials of batch A used and annual factor VIII consumption achieved significance only at the 10% level (two-tailed test). In the circumstances of this study it could be argued that one-tailed tests would be reasonable for these variables, in which case significance at the 5% level would be achieved. The fitted equation with all three terms included was:

$$P = \frac{\exp(0.83 - 2.32 \text{ Th/Ts} + 0.074 n + 0.034 \text{ ann})}{1 + \exp(0.83 - 2.32 \text{ Th/Ts} + 0.074 n + 0.034 \text{ ann})}$$

where n is the number of vials of batch A and ann the total annual usage of factor VIII (in thousands of units). Eight of nine patients with a helper/suppressor ratio of ≤ 1.5 seroconverted compared with six of fifteen with higher ratios; eight of nine patients using more than 40 vials of batch A seroconverted compared with seven of twenty-three with lower usage; eleven of thirteen patients using more than 75 000 units of factor VIII seroconverted compared with four of nineteen using less.

All patients are clinically well; two patients have persistent splenomegaly which appeared after the infusion of batch A.

LYMPHOCYTE SUBSETS OF PATIENTS WHO RECEIVED BATCH A FACTOR VIII AND FACTOR IX*

Batch and recipients	n	Mean (n) (range)					
		T helper		T suppressor		Th/Ts	
		1983	1984	1983	1984	1983	1984
Batch A, factor VIII							
HA and VWD, seroconverted	15	0.64† (14) (0.32-1.45)	* 0.62† (15) (0.14-1.47)	0.54 (14) (0.10-0.90)	0.70 (15) (0.28-1.80)	1.51† (14) (0.58-3.10)	1.14† (15) (0.27-3.30)
HA and VWD, no seroconversion		0.84 (10) (0.53-1.45)	0.77† (15) (0.30-1.42)	0.46 (10) (0.22-1.23)	0.54 (15) (0.28-0.90)	2.11 (10) (1.18-2.95)	1.60 (15) (0.33-2.40)
Batch C, factor IX							
HB, seronegative	8	0.86 (4) (0.47-1.36)	0.78 (3) (0.78-0.79)	0.56 (4) (0.36-0.87)	0.48 (3) (0.39-0.52)	1.70 (4) (0.73-2.50)	1.67 (3) (1.50-2.00)
HA with inhibitors, seronegative	2		0.70, 0.93				
Normal male controls	22	** 1.09 (22) (0.6-2.01)		** 0.56 (22) (0.22-1.21)	0.51, 0.73	** 2.05 (22) (1.59-3.93)	0.96, 1.83

HA = haemophilia A; HB = haemophilia B; VWD = von Willebrand's disease.
 *Batch C, derived from the same source plasma as batch A.
 †p<0.005 for difference between patients and controls.
 ‡p<0.005 for difference between patients who did and did not seroconvert.

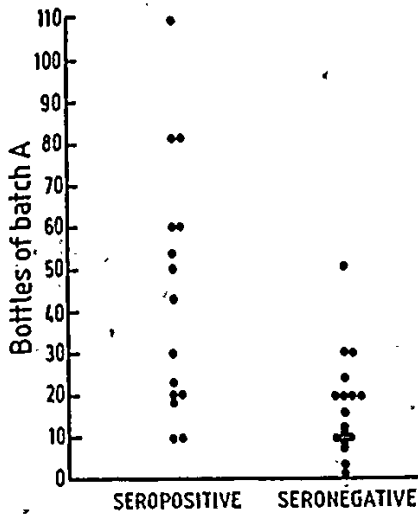


Fig 2—Number of vials of batch A of factor VIII transfused in patients who seroconverted and those who remained seronegative.

In one of these patients, reported in detail elsewhere,⁶ a glandular-fever-like illness developed, with lymphadenopathy, splenomegaly, grossly atypical peripheral blood lymphocytes, and appearance of anti-HTLV-III after transfusion of batch A.

The batch of SNBTS factor IX concentrate (C), prepared from the same source plasma as batch A was identified, and transfusion records revealed that it had been given to ten

patients, eight with haemophilia B and two with haemophilia A with anti-factor VIII inhibitors. All ten patients have been tested at least 4 months after therapy and none has detectable antibody to HTLV-III. Lymphocyte subset data were available on only small numbers and no conclusions can therefore be drawn. The average number of vials transfused per patient was only 12.5 (range 3–21).

Discussion

Scotland is one of the few parts of the western world where the frequency of anti-HTLV-III antibody in patients with haemophilia A at the beginning of 1984 was low. Until then the development of anti-HTLV-III in our patients could be attributed to the occasional use of commercial blood products in a few patients; no cases were attributable to SNBTS factor VIII. This low prevalence of antibody contrasts with that in north America. Anti-HTLV-III antibodies began to appear in haemophiliacs in 1978 in the United States⁷ but did not appear until 1979 in English haemophiliacs. During the past 7 years there has been a steady increase in the prevalence of antibodies so that now over 90% of some haemophilia populations have anti-HTLV-III antibodies.⁷

An important feature of our study is that the patients' lymphocyte subsets were measured during the spring of 1983 when all those who had received exclusively SNBTS factor VIII were negative for anti-HTLV-III. We have thus been able to compare lymphocyte subset data before and after infection with HTLV-III. It is commonly assumed that the reduction in T-helper-cell numbers is a result of the HTLV-III virus being tropic for T-helper cells.⁸ Our finding in this study that T-helper-cell numbers and the helper/suppressor ratio did not change after infection supports our previous conclusion that the abnormal T-lymphocyte subsets are a result of the intravenous infusion of factor VIII concentrates per se, not HTLV-III infection. It is possible, however, that there will be a progressive time-dependent fall in T-helper-cell numbers as a result of HTLV-III infection; but only long-term follow-up will reveal this.

Analysis of the data on this relatively small number of patients showed that the chance of developing anti-HTLV-III is dependent upon the helper/suppressor ratio, the number of transfused vials of presumed HTLV-III-infected factor VIII, and the total annual consumption of factor VIII. Our data show low helper-cell counts and helper/suppressor ratios in 1983, when the patients were negative for anti-HTLV-III and that those with helper/suppressor ratios <1.5 were more susceptible to infection; this finding supports the hypothesis of Levy and Ziegler⁹ that infection by an AIDS virus could be considered as an opportunistic infection in an immunomodulated host.

If it is true that all but one of our seropositive patients developed anti-HTLV-III as a result of the transfusion of a single contaminated batch of factor VIII, it is interesting that only half the patients who received this batch of factor VIII concentrate acquired the antibody. Possible explanations are that the apparently seronegative patients had developed antibody below the level of detection or that because of their abnormal immunological status they did not produce specific antibodies readily. A negative antibody test may reflect the absence of viral infection or replication in the lymphocytes. To determine the true viral status of these anti-HTLV-III-negative haemophiliacs we would have had to attempt viral identification or isolation from their lymphocytes.

The pool of source plasma from which the implicated batch of factor VIII was prepared was identified by the SNBTS.

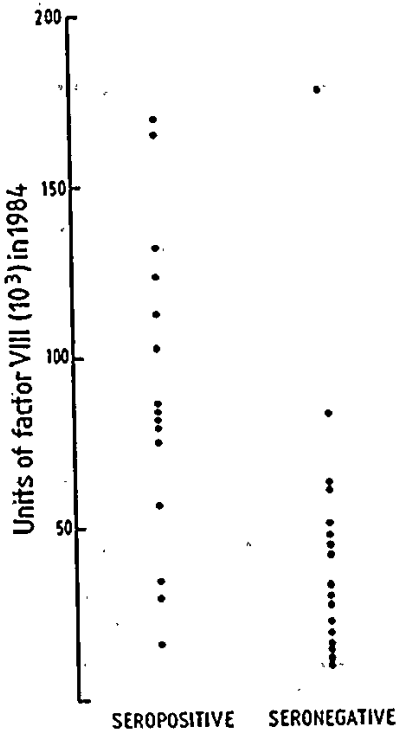


Fig 3—Total factor VIII consumption during 1984 of patients who seroconverted and those who remained seronegative.

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The factor IX batch (C) prepared from the same pool of plasma had been given to eight patients with haemophilia B and two with haemophilia A with anti-factor-VIII inhibitors. None of these individuals showed seroconversion when tested up to 4 months after infusion of this batch. Patients with haemophilia B have fewer lymphocyte subset abnormalities, a lower prevalence of antibodies to HTLV-III, and are less likely to develop AIDS than those with haemophilia A.¹⁰ It is possible that the HTLV-III virus is preferentially excluded from the factor IX concentrate during its manufacture.

The Scottish Protein Fractionation Centre has developed a programme to study possible methods for preventing the transmission of viral infections by blood factor concentrates. Although this project was initially conceived to reduce the risk of hepatitis transmission, the expertise developed was put immediately into effect after the finding of HTLV-III antibodies in our Scottish patients. All SNETS factor VIII concentrates are now heat treated. It is hoped that this will eliminate further HTLV-III infection, but only close follow-up of the patients will substantiate this expectation.

We thank Dr B. Bennett, Dr C. D. Forbes, Dr G. R. Tudhope, and Dr A. Sharp, for kindly allowing us to include patients under their care, and Mrs P.

Stewart, for typing the manuscript. This work was supported by the Medical Research Council and the Cancer Research Campaign.

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REFERENCES

1. Curran JW, Lawrence DN, Jaffe H, et al. Acquired immunodeficiency syndrome (AIDS) associated with transfusions. *N Engl J Med* 1984; 310: 69-75.
2. Editorial. Blood transfusion, haemophilia, and AIDS. *Lancet* 1984; ii: 1433-34.
3. Evans BL, Gompers ED, McDougal JS, et al. Coincidental appearance of LAV/HTLV-III antibodies in haemophiliacs and the onset of the AIDS epidemic. *N Engl J Med* 1985; 312: 483-86.
4. Carr R, Edmond E, Prescott RJ, et al. Abnormalities of circulating lymphocyte subsets in haemophiliacs in an AIDS-free population. *Lancet* 1984; i: 1431-34.
5. Cheinsoong-Popov RC, Weiss RA, Dalglish A, et al. Prevalence of antibody to human T-lymphotropic virus type III in AIDS and AIDS-risk patients in Britain. *Lancet* 1984; ii: 477-80.
6. Tucker J, Ludlum CA, Craig A, et al. HTLV-III infection associated with glandular fever-like illness in a haemophiliac. *Lancet* 1985; i: 585.
7. Kitches LW, Baria F, Sullivan JL, et al. Aetiology of AIDS-antibodies to human T-cell leukaemia virus (type III) in haemophiliacs. *Nature* 1984; 312: 347-69.
8. Schapbach J, Popovic M, Golden RV, et al. Serologic analysis of a subgroup of human T-lymphotropic retroviruses (HTLV-III) associated with AIDS. *Science* 1984; 224: 503-05.
9. Levy JA, Ziegler JL. Acquired immune deficiency syndrome (AIDS) is an opportunistic infection and Kaposi's sarcoma results from secondary immune stimulation. *Lancet* 1983; ii: 78-81.
10. Centers for Disease Control. Update: acquired immunodeficiency syndrome (AIDS) among patients with haemophilia—United States. *MMWR* 1984; 33: 589-91.

ENDOMETRIOSIS AND OVULATORY DISORDER: REDUCED FERTILISATION IN VITRO COMPARED WITH TUBAL AND UNEXPLAINED INFERTILITY

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Summary In-vitro fertilisation (IVF) was carried out once for each of 104 couples who had a single cause of infertility. The group with tubal damage was used as the reference for normal fertilising capacity of both oocytes and sperms: the IVF rates were 68% (71/105) per mature oocyte and 88% (37/42) for couples from whom mature oocytes were recovered. Couples with poor sperm/mucus penetration had reduced IVF rates: 32% (12/38) per oocyte and 60% (9/15) per couple. Sperm function, which was judged normal by means of standard seminal analysis and mucus penetration, was confirmed by normal IVF in unexplained infertility: 63% (37/59) per oocyte and 90% (18/20) per couple. Despite favourable sperm function in their partners, women with endometriosis (without tubal damage) had reduced IVF rates: 33% (19/58) per oocyte and 60% (9/15) per couple. These findings indicate that ovulatory disorder is present in endometriosis and suggest that it causes the associated infertility.

Introduction

ENDOMETRIOSIS is a well established cause of infertility (or strictly subfertility), and the association is evident even in the absence of damage to the fallopian tubes and ovaries.¹ However, the causal link is unknown and treatment is of unproven value.² Autoimmune reactions,³⁻⁵ accentuated macrophage activity,⁶⁻⁸ and prostaglandin release have been reported as local interfering effects of endometriosis, though

the last of these is controversial.⁹ Another hypothesis is that endometriosis and infertility are consequences of ovulatory disorder: the pelvic peritoneum may lose its protection against implantation of endometrial cells that reach it by retrograde menstruation, due to lack of the follicular hormones which results from failure of the follicle to rupture, despite ripening and luteinisation.^{10,11}

We have found the in-vitro fertilisation rate per oocyte in couples with chronic unexplained infertility to be slightly but significantly reduced.¹² Further analysis of the data suggested that the reduction was limited to couples with minor endometriosis, which had been ignored because of its uncertain importance. We now have sufficient findings to be conclusive.

Patients and Methods

As part of a research programme approved by the Ethics Committee of the Bristol and Weston Health District, infertile couples were offered a single attempt at in-vitro fertilisation (IVF) and embryo replacement when the woman required a routine diagnostic laparoscopy, irrespective of the results of previous investigations.

All women in the study had normal ovarian cycles, within 3-6 weeks duration and with mid-luteal progesterone levels of >30 nmol/l,¹³ and the men had normal semen by standard analysis. A motile normal sperm density of at least 4×10^6 /ml (mean of at least 2 samples) was defined as normal (product of sperm density, proportion with forward progression, and proportion with normal morphology¹⁴).

Only couples with an isolated cause of infertility in the following mutually exclusive groups were studied. (1) Tubal damage was defined by laparoscopic evidence of bilateral tubal occlusion or damage clearly interfering with oocyte pick-up, due to inflammatory disease but not endometriosis. Patients with damage due to endometriosis were excluded. (2) Failure of sperm/mucus penetration was defined by a negative^{14,15} or poor^{12,16} result on postcoital testing (PCT) in at least 2 cycles when the cervical mucus was fully developed. Women in whom mucus did not develop fully were excluded. A positive PCT (present in all the other groups) was defined by at least 1 forward-progressing sperm in every high-power-microscope field ($\times 400$) about 12 h after coitus. (3) Unexplained infertility was defined by the presence of a normal ovarian cycle, healthy pelvic organs at laparoscopy, normal seminal