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A Digital Method of Sperm Immobilization Test: Comparison to the Conventional Method

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Antisperm antibodies have been found in infertile patients and those causing immobilization of sperm are considered to be closely related to unexplained infertility. These antibodies are usually identified by a sperm immobilization test which involves counting motile sperm under microscope. This test is subjective as it relies on the judgement of the examiner with respect to sperm motility. In this study, we analyzed motile sperm by a digital method using Sperm Quality Analyzer. The results were compared with those obtained by the conventional method. We found that the two methods yielded identical results, with 14 of 66 samples tested being positive and 52 negative for sperm immobilizing antibodies. These results show that the digital method is objective and of value in the measurement of motile sperm in determination of sperm immobilizing antibodies.

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INTRODUCTION

As antisperm antibody in the sera was identified in infertile men[1], the correlation of antisperm antibody and infertility has been studied.[2] Although many assays have been used for detection of antisperm antibodies, the standardized and universally accepted assay for antisperm antibody has not been established. For example, sperm agglutination test has been used commonly for detecting antisperm antibody, however it was not significant related to infertility.[3] Recently the indirect immunobeads test has become the most popular and several studies demonstrated the close relation of antisperm and male infertility.[2–4] However, this assay is not suitable for infertile women.

Sperm immobilizing (SI) antibodies are identified in unexplained infertile patients and believed to be closely related to infertility, especially female infertility.[5] These antibodies are analyzed by a sperm immobilization test (SIT) which involves counting motile sperm under microscope.[6] The drawback of this method is that it relies on subjective judgement of sperm motility by an operator and consequently the results may vary depending on the experience and ability of the operator. In this study, we analyzed sperm immobilizing antibodies using Sperm Quality Analyzer™ (SQA™, United Medical Systems Inc., Santa Ana, CA, USA) which employs a digital method to count motile sperm. The SQA™ is a simple and inexpensive commercial unit.[7,8] We report a comparison of the results obtained by SQA™ and the conventional manual counting method.

MATERIALS AND METHODS

Subjects

Blood samples were collected from 66 infertile female patients in our infertility clinic after acquiring

TABLE I. Comparison of Sperm Immobilization Value between Conventional Method and Digital Method

	SIV	
Patient number	Conventional method	Digital method
1	1.3	1.1
2	1.1	1.3
3	1.0	0.6
4	1.0	1.1
5	1.0	0.8
6	1.0	0.7
7	1.0	0.6
8	1.3	1.7
9	1.1	1.5
10	1.0	1.4
11	1.0	1.5
12	1.0	1.3
13	1.0	1.2
14	1.0	1.2
15	1.0	0.9
16	1.0	1.0
17	1.0	1.0
18	1.0	1.3
19	1.0 (1.1)[a]	2.8 (1.3)[a]
20	1.0	0.7
21	1.0	1.4
22	1.0	1.0
23	1.0	1.8
24	1.1	1.1
25	1.0	1.5
26	1.3	1.1
27	1.1	1.3
28	1.2	1.0
29	1.2	1.4
30	0.9	1.0
31	1.2	1.1
32	1.1	0.8
33	0.9	1.3
34	1.4	0.7
35	0.9	1.3
36	0.9	1.2
37	1.4	1.2
38	1.2	0.8
39	1.5	1.6
40	1.9	1.1
41	1.4	1.3
42	1.8	0.9
43	1.3	0.7
44	1.2	1.3
45	0.8	1.1
46	0.9	1.2
47	0.9	0.8
48	1.2	0.9
49	1.3	1.4
50	1.2	1.3
51	1.1	1.0
52	2.2	2.5
53	4.7	5.2
54	2.0 (1.5)[a]	1.6 (1.6)[a]
55	∞	∞
56	∞	∞
57	20.0	∞
58	28.7	∞
59	16.0	∞
60	14.0	∞
61	∞	∞
62	∞	∞
63	∞	∞
64	∞	∞
65	∞	∞
66	23.3	24.0

SIV: sperm immobilizing value.
[a]*Result of re-examination.*

informed consents. Serum was prepared by centrifugation of the blood at 1500 g for 5 min at room temperature, and then incubated at 56°C for 30 min to inactivate complement.

Semen Analysis by SQA^{TM}

To measure the number and motility of sperm by SQA^{TM}, 20 μL of patient's serum, 2 μL of human spermatozoa suspension (4×10^7/mL) and 4 μL of complement (Low-Tox guinea-pig complement, CADARLANE Lab. Lit. Homby, Ontario, Canada) were mixed, incubated at 34°C for 60 min, and applied to SQA^{TM} according to the manufacturer's protocol.

Sperm Immobilization Test

A modified micro SIT was used.[9] Briefly, 10 μL of serum was placed in a well of Terasaki plate (Falcon No. 3034) and overlaid with paraffin oil. Two μL of guinea-pig serum as a complement source (CH50: 640) and 1 μL of active human spermatozoa suspension (4×10^7/mL) were added, and after incubation at 34°C for 60 min, motile sperm were counted under a microscope. As a complement control, 2 μL of heat-inactivated (56°C, 30 min) guinea-pig serum was used. The ratio of sperm motility with inactive complement/sperm motility with active complement was used as sperm immobilization value (SIV).[5] If the

TABLE II. Comparison of 50% Sperm Immobilization Unit (SI50) between Conventional Method and Digital Method

Patient number	Conventional method	Digital method
64	425.0	560.0
55	104.0	101.0
65	104.0	122.0
58	70.0	91.0
59	36.2	26.0
60	25.8	33.0
62	18.2	24.0
61	15.5	8.1
63	7.4	16.5
57	6.5	5.7
56	3.8	5.0
66	2.8	3.2
53	1.9	1.8
52	1.2	1.3

ratio was 2 or higher, the SIT was judged as positive. SI50 was defined as the SI titer yielding 50% sperm immobilization.[6,10]

Statistical Analysis

The results obtained by the digital and conventional methods were analyzed for correlation using software, StatView™ (Abacus Concepts Inc, Berkley, CA, USA).

RESULTS

Both the conventional and digital methods showed that 14 of 66 samples tested were positive and 52 samples negative for sperm immobilizing antibodies (Table I). Two samples (patient No. 19 and 54) gave conflicting results in the first test, but the second test yielded the same results. Fourteen samples were randomly selected and their SI50 values were estimated based on the results of the conventional and digital methods (Table II). A significant correlation was found between the SI50 values obtained by these two methods ($P < 0.0001$) (Fig. 1).

DISCUSSION

A number of investigators have reported the presence of antisperm antibodies in infertile patients. Accumulated evidence suggests that sperm immobilizing antibodies are closely related to infertility. However, the function of these antibodies is still controversial. One major reason is that there has been no standard method for identifying antisperm antibodies related to infertility. In our clinic, we employ a SIT method which involves counting motile sperm under microscope.[5,11] In this study, we used a digital method using SQA™ which should provide objective analysis of sperm motility as compared with the conventional sperm analysis. We found that there was a significant correlation in the assay results of sperm immobilizing antibodies between the conventional method and the digital method using SQA. Therefore, SQA should provide as useful a method as the conventional method performed by an experienced operator.

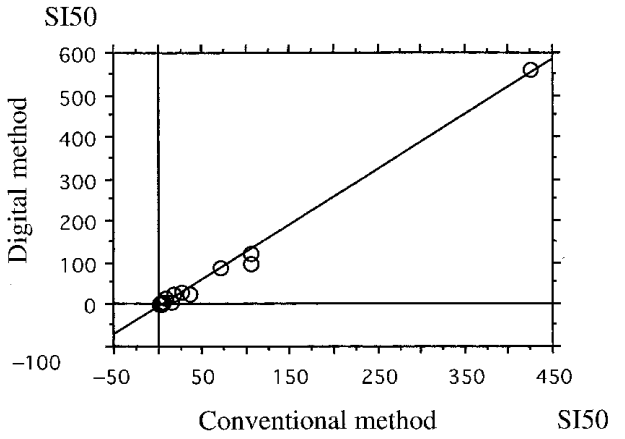

Fig. 1. Correlation of SI50 values obtained by the conventional and digital methods. Data presented in Table II are used in this figure.

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