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Efficacy of the sperm survival test for the prediction of oocyte fertilization in culture

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The present study was carried out to investigate the predictive value of the sperm survival test (SST) with respect to the fertilization of oocytes in culture. In general, our laboratory uses a total of 50 000–150 000 motile spermatozoa to inseminate each oocyte. The remaining material is evaluated for motility before and after 24 h of incubation at 37°C in a 5% CO_2 atmosphere. A total of 250 oocytes from 50 cases (mean ± SD, 5.0 ± 2.4 oocytes per retrieval) were inseminated and the final rate of cleaved embryos obtained was 52.5%. The SST (%) was considered normal when the ratio (final density of progressing spermatozoa after 24 h × 100/initial density of progressing spermatozoa) was 50% or more. Any other result was considered abnormal. Cases presenting one or more cleaved embryos (n = 40) were separated from those in which no embryo formation occurred (n = 10) and the results were compared in terms of the respective sperm survival rates over a period of 24 h: normal SST (one or more cleaved embryos, 37; none, five), abnormal SST (one or more cleaved embryos, three; none, five). The specificity of the SST was 0.92 and sensitivity 0.50, the predictive value of the abnormal test was 0.62 and the predictive value of the normal test 0.88. The efficacy of the test was estimated at 0.71, which was better than the conventional parameters of sperm analysis. A receiver–operating characteristics curve for SST confirmed that the test can be useful for the prediction of fertilizability of oocytes in the laboratory.

Key words: IVF/prediction of oocyte fertilization/sperm survival test

Introduction

The biological activity of spermatozoa can be assessed by several methods in the laboratory, including their ability to migrate into the cervical mucus (Barratt *et al.*, 1989), or of tail swelling in hypo-osmotic solution (Jeyendran *et al.*, 1984), to penetrate zona-free hamster oocytes (Stenchever *et al.*, 1982), or to bind to the zona pellucida isolated from human oocytes (Coddington *et al.*, 1990). In combination, these assays indicate the ability of spermatozoa to pass through the cervix, to undergo capacitation, to acrosome-react, to bind to the zona pellucida, to fuse with and penetrate the vitelline membrane, and to decondense after entry into the ooplasm. Thus, combined, the tests should be a fairly good indication of the fertilizing capacity of spermatozoa.

The sperm survival test (SST) is also frequently used for quality control in *in vitro* fertilization (IVF) laboratories (Purdy, 1982; Critchlow *et al.*, 1989). However, there are no references about the usefulness of the test as a method to predict oocyte fertilization in culture. Since the SST is simple and easily executed, in the present study we tested its efficacy for evaluation of the biological activity of spermatozoa in the laboratory.

Materials and methods

The material consisted of 50 sperm samples from 50 men who participated in the IVF programme of the Human Reproduction Centre, 'Sinhá Junqueira' Maternity Foundation, together with their wives. The samples were collected by masturbation in sterile plastic containers after a period of at least 2 days of sexual abstinence. The semen samples were allowed to liquefy completely and the following semen characteristics (normal values in parentheses) were measured by standard techniques: sperm concentration ($\geq 20 \times 10^6$ per ml), normal forms ($\geq 30\%$), and progressive motility ($\geq 30\%$).

Spermatozoa were prepared by the swim-up technique using Menezo B2 medium enriched with 30% inactivated serum in a 3:1 ratio. After two successive centrifugations at 200 *g* for 5 min, 0.3–0.5 ml of medium was added to the pellet and the material was incubated for 45 min. The supernatant was then removed and a final concentration of 500 000 to 1.5×10^6/ml motile progressive spermatozoa was obtained with the aid of a Makler chamber (Sefi-Medical Instruments, Haifa, Israel).

Of the 50 patients studied, age range 21–45 years (mean ± SD, 31.2 ± 4.7 years), 29 reported primary infertility and 21 secondary infertility. Ovarian stimulation was performed in a cycle semi-programmed with pills followed by clomiphene citrate, gonadotrophins and dexamethasone in 40 patients, and with analogues followed by gonadotrophins in the remaining 10. In general, on the eighth day of stimulation, the patient was evaluated for follicular development by ultrasound, using a 5.0 MHz vaginal transducer. When a minimum of two or more 17 mm diameter follicles were obtained, and in the absence of an endogenous surge of luteinizing hormone (LH), human chorionic gonadotrophin (HCG) was injected i.m. at a dose of 10 000 IU. Aspirative puncture was performed 34–36 h after HCG administration; the oocytes identified in the follicular fluid were classified for maturity and placed on Nunc plates (Nunclon,

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Denmark) containing Menezo B2 (Apy-System, Montalieu-Vercieu, France) enriched with 10% patient serum. At 4–6 h after oocyte collection, 100–200 μl of washed spermatozoa was used for oocyte insemination.

The remaining volume was then checked for initial concentration of motile spermatozoa and incubated for 24 h in 5% CO_2. The final concentration of motile progressive spermatozoa was then determined and 24 h SST was calculated as follows:

$$\text{SST (\%)} = \frac{\text{final concentration of progressive spermatozoa after 24 h} \times 100}{\text{initial concentration of progressive spermatozoa}}$$

The 24 h SST was considered to be normal when the ratio was 50% or more and was considered to be abnormal in any other case.

Embryo cleavage was observed 48 h after insemination. Statistical analysis was performed by calculating the sensitivity, specificity, efficacy (sensitivity + specificity/2), predictive values of abnormal and normal test results, and false positive rate (1 − specificity) (Stempel, 1982; Peng *et al.*, 1987). A number of cut-off points defining the values were selected to construct a receiver–operating characteristics curve (ROC) for the SST (Peng *et al.*, 1987).

Results

A total of 76% of the initial sperm samples were considered to be within the normal limits of concentration, motility and morphology adopted in our laboratory. Mean 24 h sperm survival was 62.2% (range, 0–100%). The SST was normal in 42 cases and abnormal in eight.

A total of 250 oocytes from 50 patients (mean ± SD, 5.00 ± 2.41) were inseminated, and a mean embryo cleavage rate of 52.5% (range, 0–100%) was obtained after 48 h. A total of 40 patients presented one or more cleaved embryos, and no cleavage occurred in 10 cases (Table I).

Table I. Comparison between the results of the sperm survival test and the presence or absence of cleaved embryos

Sperm survival test	Embryos	
	one or more	none
Normal	37	5
Abnormal	3	5

Table II. Statistical properties for conventional parameters of sperm analysis and sperm survival test for the prediction of oocyte fertilization at the normal cut-off point (50%)

Statistical properties	Concentration 20×10^6/ml	Morphology 30% normal	Motility 30%	Sperm survival test 50%
Sensitivity	0.20	0.62	0.20	0.50
Specificity	1.00	0.70	0.70	0.92
Efficacy	0.68	0.66	0.45	0.71
APV	1.00	0.29	0.14	0.62
NPV	0.88	0.90	0.77	0.88
False positive	0	0.30	0.30	0.08

APV = predictive value of an abnormal test.
NPV = predictive value of a normal test.

Table II shows the sensitivity, specificity, efficacy, the predictive values for abnormal and normal test results, and false positive rate (1 − specificity) for the conventional parameters of sperm analysis (density, morphology, motility) compared to the values obtained by the SST at a normal cut-off point (50%). Table III contains the statistical properties for SST calculated at various cut-off points. Receiver–operating characteristics (ROC) curves described by Peng *et al.* (1987) were then constructed for SST (Figure 1).

Discussion

In general, the usual parameters of sperm analysis do not provide precise criteria for assessing male factor performance in a programme of IVF (Hewitt *et al.*, 1987). This was confirmed in a study of hormonal parameters by Ramsewak *et al.* (1990), who observed that for a total of 152 oocytes collected in an IVF programme employing only natural and clomiphene citrate-induced cycles, pre-ovulatory follicular fluid steroids, pro-

Table III. Statistical properties of the sperm survival test calculated at various cut-off points

Statistical properties	Cut-off points (ratios of sperm survival test)				
	20	40	50	60	90
Sensitivity	0.30	0.40	0.50	0.60	0.90
Specificity	0.97	0.92	0.92	0.85	0.35
Efficacy	0.63	0.66	0.71	0.72	0.62
APV	0.75	0.57	0.62	0.45	0.25
NPV	0.84	0.86	0.88	0.87	0.93
False positive	0.03	0.08	0.08	0.15	0.65

APV = predictive value of an abnormal test.
NPV = predictive value of a normal test.

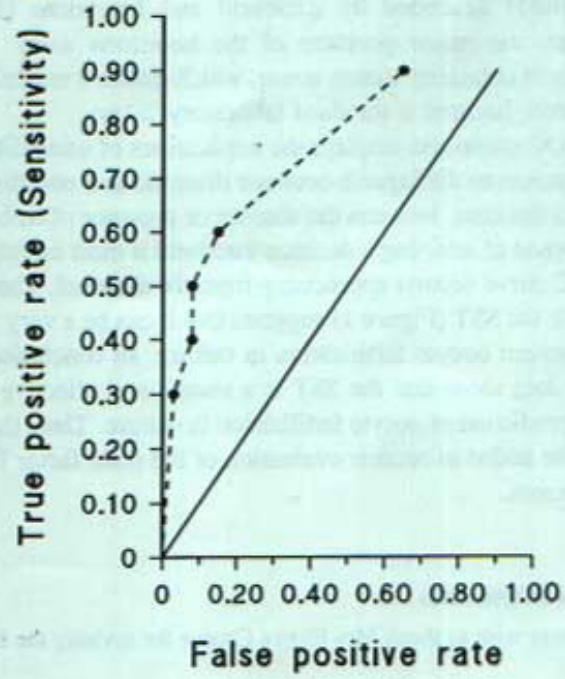

Fig. 1. Receiver–operating characteristics curve plotted from the true positive rate (sensitivity; dashed line) and false positive rate (1 − specificity; solid line) obtained for the sperm survival test carried out on 50 patients.

gesterone, oestradiol, oestradiol:progesterone ratio, and LH, as well as baseline plasma LH and the magnitude of the LH surge did not correlate with fertilization.

Usually, in order to compensate for this lack of predictability of fertilization of oocytes in culture, biological assays are used to provide more data, especially with respect to the presence or absence of alterations of the male factor in assisted reproduction. Unfortunately, these biological assays are not always easy to perform and biological material is sometimes difficult to obtain.

Barratt *et al.* (1989) showed that the sperm–mucus penetration test may be of some predictive value in the determination of IVF of human oocytes, with no false positive results, i.e. no fertilization occurred when mucus penetration was not observed. In the same study, the hypo-osmotic test proved to be of low predictive value.

In the present study, statistical analysis (Table II) demonstrated that the SST presented acceptable predictive values for oocyte fertilization (presence or absence of embryos) both for normal (0.88) and abnormal (0.62) tests when compared to those obtained with sperm concentration (normal, 0.88; abnormal, 1.00), morphology (normal, 0.90; abnormal, 0.29), and motility (normal, 0.77; abnormal, 0.14). The efficacy of SST (0.71) was also higher than that of conventional parameters of sperm analysis. Furthermore, the SST performed after 24 h of culture showed a specificity of 0.92, similar to the 0.97 value observed for the hamster test by Stenchever *et al.* (1982) in an identical experimental situation.

According to Acosta (1992), the hamster test correlates well with the results of IVF when the test is normal. However, spermatozoa from a total of 65.8% of the patients whose tests were abnormal succeeded in fertilizing oocytes *in vitro*. These conclusions are similar to those obtained here for the SST, i.e. high specificity (0.92) and discrete sensitivity (0.50). The efficacy of the SST was slightly lower (0.71) than that of the hemizona assay (0.81) described by Zaneveld and Jeyendran (1992). However, the major problem of the hemizona assay is the difficulty in obtaining human zonae, which makes it unlikely that it will ever become a standard laboratory assay.

An ROC curve also displays the implications of using different cut-off points to distinguish between diseased and non-diseased states (in this case, between the absence or presence of embryos). This method of selecting a decision threshold is most useful when the ROC curve departs appreciably from the diagonal. The ROC curve for the SST (Figure 1) suggests that it can be a very useful test to screen oocyte fertilization in culture. In conclusion, the present data show that the SST is a simple and effective assay for the prediction of oocyte fertilization in culture. Thus, the SST should be added to routine evaluation of the male factor in IVF programmes.

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