Human Reproduction vol.12 no.9 pp.1969–1973, 1997

A sperm survival test and in-vitro fertilization outcome in the presence of male factor infertility

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Several tests based on semen variables have been proposed to predict the fertilization rate in the presence of male factor infertility, but their significance remains unclear. We investigated the utility of a screening test based on sperm survival (SST) to predict the outcome of in-vitro fertilization (IVF) cycles in the presence of male factor infertility. The SST was considered normal when the percentage of motile spermatozoa 24 h after oocyte insemination was ⩾50%. The sperm survival test yielded abnormal results in <90% of cycles which were unsuccessful. The sensitivity of the SST was 87% and specificity 65% with a positive predictive value of 90% in the male factor group. We believe that the SST may be a useful predictor of the IVF cycle outcome and we propose its introduction into the routine preliminary evaluation of semen samples in cases of male factor infertility.

Key words: in-vitro fertilization/male factor infertility/prediction of oocyte fertilization/sperm survival test

Introduction

It is well known that the success rate of in-vitro fertilization (IVF) cycles is lower in the presence of male factor infertility (Cohen *et al.*, 1984; Acosta, 1992). In spite of extensive studies performed to evaluate the fertilizing potential of spermatozoa, the clinical application of the several tests designed remains controversial. Sperm survival is currently defined as the ability to maintain motility over time. The sperm survival test (SST) is frequently used for quality control in IVF laboratories (Purdy, 1982; Critchlow *et al.*, 1989) but is not included in the screening of patients undergoing IVF programmes. Since it is conceivable that such a parameter may be highly relevant in determining the likelihood of success in IVF, particularly in cases of male factor infertility (Franco *et al.*, 1993; Stovall *et al.*, 1995), we investigated the utility of a screening test based on sperm survival (SST), as an adjunct to standard evaluation according to World Health Organization criteria, in predicting the outcome of IVF cycles for male factor infertility.

Materials and methods

All patients undergoing IVF for male factor infertility at the Department of Reproductive Medicine of the University of Florence from September 1992 to March 1994 were prospectively recruited for our analysis. As a control group, a further 81 cycles with tubal infertility and normal semen parameters were studied. The 160 cycles for male factor infertility were divided into three groups according to semen parameters: group I, cycles whose sperm concentration was $<20\times10^6$/ml and defined as oligospermia; group II, cycles whose percentage progressive motility was <35% and defined as asthenospermia; group III, cycles whose sperm concentration was $<20\times10^6$ /ml and percentage progressive motility was <35% and defined as oligoasthenospermia. In order to select a group of severely asthenospermic patients where the reduced motility could have an influence upon the fertilization rate, a more strict criterion was employed than that proposed by the World Health Organization (WHO) (50% progressive motility) (WHO, 1992). Thus, in groups II and III, only cycles with a percentage progressive motility <35% were considered.

All patients underwent controlled ovarian hyperstimulation for in-vitro fertilization (IVF) and embryo transfer. Patients received the gonadotrophin-releasing hormone analogue (GnRHa) buserelin (Suprefact; Hoechst A.G., Germany) 0.3 ml s.c. twice daily from mid-luteal phase until pituitary desensitization was achieved. Multiple follicular development was induced with three ampoules of human menopausal gonadotrophin (HMG 75 IU; Pergogreen, Serono, Rome, Italy). Ovarian stimulation was monitored by oestradiol determinations, and transvaginal scanning (Aloka SSD 630, Tokyo, Japan) of the ovaries starting on day 6 of gonadotrophin administration. Ovulation was induced with human chorionic gonadotrophin (HCG) 10 000 IU i.m. (Profasi 5000; Serono) in the presence of two follicles ⩾18 mm and oestradiol values >800 pg/ml. After 36 h, ultrasound-guided retrieval of oocytes was performed. Luteal phase was supported by 50 mg of progesterone i.m. (Gestone Pabyrn; AMSA, Rome, Italy) starting on the day of oocyte retrieval.

Semen samples were collected on the morning of oocyte retrieval. The semen was assessed for concentration, motility and morphology, according to the WHO standards (1992). The sample was diluted in a ratio of 1:2 with human tubal fluid medium (HTF, Irvine Scientific, Santa Ana, California, USA) supplemented with 1% human serum albumin (HSA, Irvine Scientific) and centrifuged at 500 *g* for 10 min. The supernatant was removed, and the pellet was resuspended in 1.5 ml medium and centrifuged again under the same conditions. A swim-up migration was then performed by placing a layer of fresh medium (1 ml) over the sperm pellet and incubating for 45 min at 37°C under an atmosphere of 5% CO_2 in air. The overlaying medium was then aspirated and sperm concentration and motility were evaluated in Mackler chamber (Sefi-Medical Instruments, Haifa, Israel).

The oocytes retrieved were classified for maturity according the criteria proposed by Veeck (1988) based on the exact nuclear condition of the oocyte (metaphase MII–MI to prophase PI) and placed in culture tubes containing 1 ml of Ménézo B_2 (Diasynt, Florence, Italy) enriched with HSA. Four to 5 h after collection, the oocytes

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Table I. The IVF success rate in male factor group according to WHO criteria

	n	Cycles with fertilization	%	Fertilization
Group 1 oligospermia	35	21	60	13.7
Group 2 asthenospermia	65	40	61.5	30.3
Group 3 oligoasthenospermia	60	19	31.7	12.2
Total male factor group	160	80	50	19.7
Control group	81	80	98.7	63.7

1 versus 2, n.s.; 1 versus 2, $P = 0.001$; 1 versus 3, $P = 0.013$; 1 versus 3, n.s.; 2 versus 3, $P = 0.001$; 2 versus 3, $P = 0.0001$; total versus control $P < 0.0001$, $P < 0.0001$.

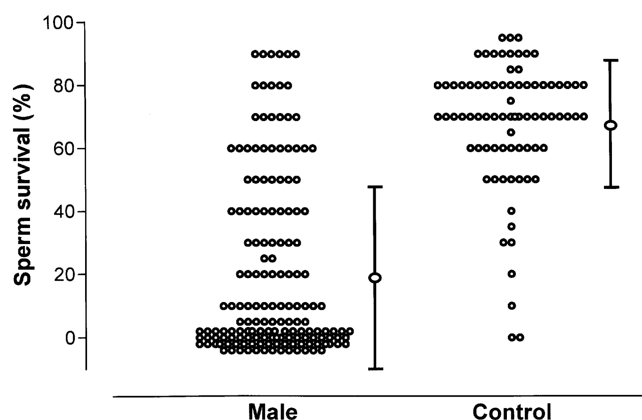

Figure 1. Comparison of sperm survival test in 160 patients with male factor infertility (male) and 81 patients with tubal factor infertility and normal semen parameters (control).

were inseminated with 100 000 motile spermatozoa when available (depending on the seminal sample). Only oocytes at MI or MII stage were included in the analysis of fertilization rate. The remaining sperm volume after oocyte insemination was then checked for concentration of motile spermatozoa and incubated for 24 h in 5% CO_2. After this time, the concentration of motile progressive spermatozoa was determined and SST was calculated as the ratio between the final concentration of progressive spermatozoa after 24 h (×100) and the initial concentration of progressive spermatozoa.

The SST was considered to be normal when the ratio was 50% or more and was considered to be abnormal in any other case. The 50% survival threshold was chosen based on published data (Franco *et al.*, 1993). Sixteen to 20 h after insemination, the oocytes were observed for the presence of two pronuclei and embryos were checked for cleavage approximately 42 h after insemination. Up to four embryos were selected for transfer using a Frydman catheter (CCD, Paris, France). Outcome parameters included percentage of cycles with fertilization and the fertilization rate.

Statistical analysis was performed on an IBM compatible computer using the SPSS for Windows (SPSS Inc., Chicago, IL, USA). Data were analysed using χ^2 or Fisher's test as appropriate to compare the percentage of cycles with fertilization and the fertilization rate in the male factor group and the control group. A non-parametric test (Mann–Whitney test) was used to compare the mean value of SST in the two groups. Probability values of <0.05 were considered significant. The calculation of sensitivity, specificity, accuracy, efficacy (sensitivity + specificity/2), predictive values of abnormal and normal test results and false positive rate (1 – specificity) of the SST was performed (Griner *et al.*, 1981). A receiver-operating characteristics (ROC) curve (Peng *et al.*, 1987) was constructed for SST in male factor and control groups at different cut-off values (0, 20, 50, 70%) based on fertilization results. We considered to be true positive those cycles with an abnormal SST result and low fertilization rate (<50%) and to be true negative those cycles with a normal SST and fertilization rate ≥50%.

A multivariate analysis was performed using the Cox regression method in order to evaluate the prediction of semen variables on the fertilization rate. The dichotomous variable fertilization rate >/<50% was included in the analysis, together with the following semen parameters: sperm concentration and progressive motility in fresh sample and motile sperm fraction, percentage of normal morphology (assessed according WHO criteria) and sperm survival.

Results

Clinical and cycle characteristics of female partners, such as patient age, number of HMG ampoules used for ovarian hyperstimulation, cycle day of HCG administration, oestrogen value and endometrium thickness on day of HCG administration, did not differ between male factor and control groups. The women in the study ranged in age from 21 to 42 years (mean 34.4 ± 4.3) in the male factor group and from 23 to 40 years (mean 33.3 ± 3.8) in the control group ($P = 0.09$). The mean number of oocytes retrieved per patient was 9.4 ± 5.7 in the male factor group and 10.3 ± 4.1 in the control group ($P = 0.3$). The percentage of oocytes at MII and MI was 67 and 70% respectively in the male factor and control groups ($P = 0.2$).

Of the 160 cycles for male factor infertility, successful fertilization was obtained in 80 (50%). In the control group of 81 cycles, fertilization was achieved in 80 cycles (98.7%) ($P < 0.0001$). The fertilization rate was 19.7% (265/1344, range 0–100%) in the male factor group and 63.7% (529/831, range 0–100%) in the control group ($P < 0.0001$). A total of 75 cycles (46.9%) and 78 cycles (96.3%) produced one or more cleaved embryos respectively in male factor group and control group ($P < 0.0001$). No cleavage occurred in five cases in the male factor group and in two cases in the control group.

The IVF success rate was expressed according to the standard WHO criteria for the evaluation of semen samples in male factor infertility (Table I).

Mean sperm survival 24 h after insemination was 21.4% (range 0–90%) in male factor group and 67.8% (range 0–95%) in control group ($P < 0.0001$) distributed as shown in Figure 1.

Table II shows the statistical properties of SST calculated at various cut-off points in male factor and control group considering true positive cycles with low fertilization rate (<50%). Table III shows the sensitivity, specificity, accuracy,

Table II. Statistical properties of sperm survival test (SST) at various cut off values in male factor and control groups

Statistical properties	Male factor group Cut-off values of SST				Control group Cut-off values for SST			
	0	20%	50%	70%	0	20%	50%	70%
Sensitivity	51	77	87	95	13	13	31	63
Specificity	79	74	65	32	100	97	95	75
Accuracy	57	76	83	82	83	80	83	73
Positive predictive value (PPV)	90	92	90	84	100	50	63	38
Negative predictive value (NPV)	30	46	58	65	82	82	85	89
Efficacy	65	76	76	49	57	55	64	69
False positive rate	21	26	35	68	0	3	5	25

Table III. Statistical properties for the ability of conventional parameters of sperm analysis and sperm survival test (SST) to predict oocyte fertilization at the cut-off value of 50%

Statistical properties	Concentration $<20 \times 10^6$/ml	Progressive motility $\leqslant$35%	SST 50%
Sensitivity	65	78	87
Specificity	62	21	65
Accuracy	64	66	83
Positive predictive value	86	78	90
Negative predictive value	32	20	58
Efficacy	64	50	76
False positive rate	38	79	35

Table IV. Logistic regression analysis: significance levels obtained for each semen variable associated with a fertilization rate >50%

Semen variable	Univariate	Multivariate
Fresh sample		
Concentration	0.006	0.22
Progressive motility	0.08	0.53
Percentage normal morphology	0.05	0.99
Motile fraction		
Concentration	0.007	0.89
Progressive motility	0.003	0.06
Sperm survival	<0.00001	0.0003

predictive values, efficacy and false positive rate for the conventional parameters of sperm analysis (sperm concentration and motility) compared to the values obtained by the SST at a cut-off point of 50% in male factor group in the presence of low fertilization rate (<50%). A univariate logistic regression analysis revealed a significant predictive value for fertilization (considering FR >50% as a positive result) for sperm concentration in fresh sample, sperm concentration and progressive motility in motile fraction and sperm survival. However, when the same variables were included in a multivariate analysis, sperm survival was the only variable independently associated with a fertilization rate >50% (Table IV).

The ROC analysis of SST at different cut-off values showed that the 50% survival threshold proved to be the best combination of sensitivity and specificity (Figure 2).

The SST was abnormal (<50%) in 122 cycles in the male factor group (76%) and in eight cycles (9.8%) in the control group ($P < 0.0001$). It yielded abnormal results in over 90% of cycles which were unsuccessful in the male factor group (Table V). When each group was separately analysed, the correlation between abnormal SST and failed fertilization was confirmed (95% in the specific case of oligoasthenospermic group). In particular, the SST was 0% (no motile spermatozoa after 24 h from oocyte insemination) in 52 cycles out of 80 without fertilization (65%), compared with 19 out of 80 (23.7%) in cycles with fertilization ($P = 0.0001$) (Table VI). In the control group we found an SST of 0% in only two cases, in one of which the patient had failed fertilization.

Discussion

In-vitro fertilization as a possible treatment for couples suffering from male factor infertility was suggested in 1984 by Cohen *et al.* Since then, male factor infertility has been recognized in several studies as a poor prognostic factor for success (Mahadevan and Trounson, 1984; Van Uem *et al.*, 1985). With the advent of assisted fertilization techniques, it has become important to differentiate between male infertility patients who may benefit from routine IVF treatment and those who require micromanipulative procedures (Ord *et al.*, 1993; Van Steirteghem *et al.*, 1993).

Several studies have reported correlations between the fertilization rate and specific semen variables, such as sperm concentration, motility and morphology, in the fresh samples or motile sperm fractions (Battin *et al.*, 1985; Aitken, 1988; Duncan *et al.*, 1993). However, such standard parameters of sperm analysis do not provide precise criteria for assessing the fertilization potential in an IVF programme for male factor infertility, with the exception of morphology evaluated with strict criteria (Kruger *et al.*, 1988). Several other tests have been introduced to define the issue further, but they have as yet either proved to have low predictive value such as the swelling test (Jeyendran *et al.*, 1984; Sjoblom and Coccia, 1989), or their use remains controversial, such as the hamster egg penetration assay (Liu *et al.*, 1989) and the hemizona assay (Burkman *et al.*, 1988; Oehninger *et al.*, 1989). Moreover, numerous biochemical determinations have been proposed in an attempt to better define sperm function such as acrosin activity measurement (De Jonge *et al.*, 1993) and adenosine triphosphate measurement (Chan and Wang, 1987), but there is still no consensus that these tests would prove useful to predict fertility potential in male factor patients. A recent report by our group described a good relationship between the responsiveness of spermatozoa (measured as increase of $[Ca^{2+}]_i$ and acrosome reaction) to progesterone *in vitro* and their

Table V. Comparison between the abnormal sperm survival test (SST) and the cycles with failed fertilization

	No. cycles	No. cycles with failed fertilization	SST $<$ 50% with failed fertilization	%
Group 1 Oligospermia	35	14	13	93
Group 2 Asthenospermia	65	25	21	84
Group 3 Oligoasthenospermia	60	41	39	95
Total male factor group	160	80	73	91
Control group	81	1	1	100

Table VI. Distribution of the sperm survival test (SST) in cycles with failed fertilization and in cycles with FR $<$50% and $\geqslant$50%

SST	Cycles with fertilization			Cycles without fertilization	Total
	Total	FR $\geqslant$50	FR $<$50%	FR=0%	
0%	19	7	12	52	71
1–50%	30	5	25	21	51
$>$50%	31	22	9	7	38
Total	80	34	46	80	160

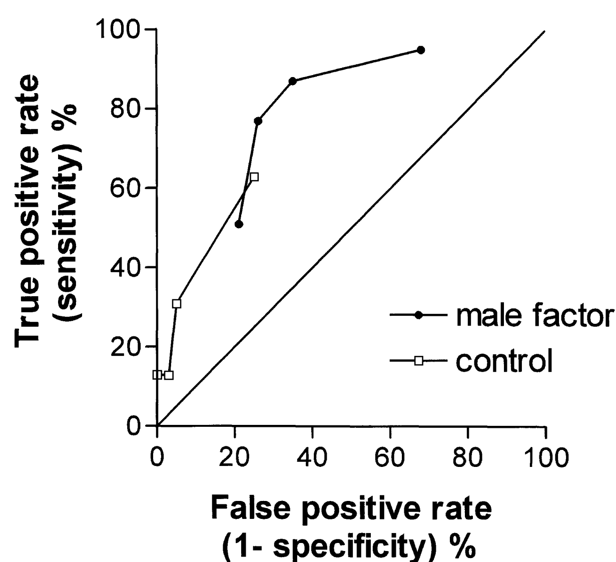

Figure 2. Receiver operator characteristics curve of SST plotted from the true positive rate (sensitivity) and false positive rate (100 – specificity) in male factor group (●) and control group (□).

fertilizing capacity (Krausz *et al.*, 1995). Several studies have evaluated sperm survival, defined as the capacity of spermatozoa to maintain motility over time, which is currently assessed in several IVF laboratories for quality control (De Ziegler *et al.*, 1987; Critchlow *et al.*, 1989). To our knowledge, Franco *et al.* (1993) were the first to investigate the utility of an SST as a method to predict oocyte fertilization in an IVF programme. However, the number of cycles analysed in this study was limited and the indication for IVF was not taken into consideration, so that the reported good specificity was outweighed by a poor sensitivity value, which is unacceptable for a potential screening test. In a further study, Stovall *et al.* (1995) reported good predictive accuracy of an SST in a large series of IVF patients. However, they included in the analysis, couples with several infertility factors. Therefore, in the present study, we chose to evaluate the predictive value of SST in adjunct to the standard WHO criteria with respect to fertilization of oocytes in a male factor group compared to a control group. The aim of the study was to test the hypothesis that accurate sperm evaluation, comprising sperm survival analysis combined with the standard WHO criteria, may allow a more appropriate selection of patients for whom routine IVF may or may not be appropriate.

Our data support the evidence, widely reported in the literature, of particularly low success rates in IVF programmes in presence of male factor infertility (20% in our study), with special regard to the oligoasthenospermic group (FR 12.2%). In all such cases, therefore, there is a substantial risk for the patients and the institution to undertake long, expensive and invasive procedures with minimal success probabilities, which are not justified on a cost–benefit ratio basis and bear negative psychological consequences for the couple. Hence, we are especially interested in developing appropriate guidelines for IVF programmes dealing with this specific problem.

When routinely examined in the prospective assessment of patients for our IVF programme, the male factor samples showed significantly reduced sperm survival as compared to normal controls [mean of 21.4% versus 67.8% in control ($P < 0.0001$)].

When a cut-off of 50% survival was considered, different results in terms of sensitivity, specificity and predictive values were obtained respectively in male factor and control groups. In the male factor group the SST showed high sensitivity (87%) with a specificity of 65%, while in the control group the sensitivity was not acceptable for a screening test (31%) even if the specificity was high (95%). Moreover, we observed in the male factor group a higher positive predictive value (90%) compared to the control group (63%), which confirmed a good correlation between a positive result of the SST and lower fertilization rates. On the other hand, the relatively low negative predictive value (58%) showed a reduced correlation

between negative results and good fertilization rates. Finally, the accuracy of SST in male factor group was higher (83%) than that of conventional parameters of sperm analysis (sperm concentration 64; motility 66%). The sensitivity and specificity of SST were also higher (87; 65%) when compared to sperm concentration (65; 62%) and motility (78; 21%). These results are supported by a multivariate analysis including all standard semen variables (according to WHO) and sperm survival, in which sperm survival was the only variable independently associated with a fertilization rate >50%.

In ROC curve analysis, the power of a test to discriminate between two populations was studied. The further the curve shifts to the upper left hand corner of the ROC plot, the better the test can discriminate between the two populations. The ROC curve for an ideal diagnostic test reaches the upper left hand corner (Peng *et al.*, 1987). In our study, the ROC curve for the SST (Figure 2) suggests that the test may prove very useful to screen oocyte fertilization in presence of male factor infertility. Regarding the different cut-off values reported in the curve, the value of 50% survival proved to be the optimal combination of sensitivity and specificity confirming previously published data (Franco *et al.*, 1993).

In conclusion, an abnormal SST was significantly associated with oocyte fertilization failure. In particular, >90% of unsuccessful IVF cycles showed abnormal sperm survival (95% in the specific case of the oligoasthenospermic group).

Therefore, we suggest the use of the SST as a routine test in the preliminary evaluation of the male factor in IVF programmes to achieve a complete assessment of the seminal parameters. Since the SST is simple and easily performed, we emphasize the routine use of this test in addition to the evaluation of the seminal parameters according to the standard WHO criteria, even in the semen samples collected on the day of oocyte retrieval.

We believe that this test could identify the group of patients with very low chances of success with routine IVF and for whom micromanipulation techniques may be indicated to avoid inappropriate use of both IVF and ICSI.

Acknowledgements

We thank Dr S.Pellegrini. Dr C.Cozzi, Dr M.Mannelli, Dr S.Pratesi and Dr I.Noci for their clinical contribution.

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Received on January 13, 1997; accepted on June 13, 1997