Fertilising capacity of human sperms: A simple predictive assay

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Male subfertility is a growing reason for assisted reproduction. A limiting factor in male subfertility is asthenospermia. Motility is a cardinal indication of sperm vitality. Thus prognostic assays are aimed at quantitative determination of progression to assess the fertilising potential. However, a method permitting reliable prognosis of the fertilising capacity has yet to be developed. The assay presented here is the outcome of empirical data based on 590 IVF (in vitro fertilisation) trials. It is essentially a further exploitation of the Swim Up procedure, the selected sperms being maintained in culture under identical conditions employed in IVF. Semi-quantitative daily recordings of linear progression until complete extinction provided an index on vitality which is directly related to the fertilising potential. The findings indicated that a threshold of 50% linear motility after 24 hr culture was required to initiate fertilisation. The fertilising potential was guaranteed when at least 60% linear motility was observed at 24 hr, making the assay a predictive one. Its simplicity is an attractive feature.

The application of reproductive technologies is a cornerstone in reproductive medicine. Before the introduction of intracytoplasmic sperm injection (ICSI) to achieve fertilisation in cases of asthenospermia and even sperm immotility, an uncompromising prerequisite has been the capability of the gametes to fertilise spontaneously. As ICSI is a last resort, laboratory tests to assess sperm motility as an indication of vitality are indispensable, since artificial insemination and IVF (in vitro fertilisation) are the predominant forms of assisted reproduction. To determine motility, in addition to spermiograms (see WHO Manual1), a variety of other complementary tests have been recommended. These, essentially, aim at quantifying sperm activity with the view to render predictability on the fertilising potential. Sperm motility is recognised as a critical parameter reflecting functional vitality and with high probability, the capacity to fertilise.3,6 This presupposes that other indispensable functions like the acrosomal reaction7,8 and the ability to bind with the zona pellucida receptor9,10 are not defective. Both are rare congenital defects.

Since the number of spermatozoa required for assisted reproduction (artificial insemination and IVF, excepting ICSI) is very small, oligospermia can generally be overcome by cell enrichment techniques. The unsurmountable factor in male subfertility is asthenospermia. When these two features occur together - which is often the case - then, artificial insemination and IVF will not be successful. Thus, the need for reliable diagnostic tests to determine the fertilising potential of sperms is of utmost importance in patient counselling and treatment.

Conventional spermiograms assess the motility by direct microscopic examination of native semen at intervals using a system of grading. The errors of judgement are reflected in the frequency of errors in prognosis. Although improvement has been achieved through the use of computer aided motility analysers11, this technology has not led to the development of a predictive assay. Kremers12 developed a method of measuring the penetration of sperms in samples of patients’ cervical mucus. A modification of the Kremers Test substitutes cervical mucus with sodium hyaluronate.13 A commercialised version of the Kremers Test employs bovine cervical mucus.

The assay presented here is based on selection (Swim Up) and culture of the sperms in standard IVF culture medium - which includes serum from the partner - followed by daily estimations of the percentage of linearly progressing sperms as a means of determining sperm vitality, an index of the fertilising potential. Analyses of the data indicated a threshold for the fertilising potential.

Native semen must be appropriately prepared before use in IVF and intrauterine insemination. Attempts have been made at improving sperm selection by various methods other than conventional washing followed by Swim Up, without appreciable success. Washing is usually done twice except when oligoasthenospermia was diagnosed, when washing was only once.
For IVF, oocytes were incubated in modified Ham's F10 medium (adjusted to 280 mOs) containing 7.5% patients' own serum, 3 to 5 hr postretrieval before addition of the husbands' sperms at about $10^5$ sperms/ml/oocyte. Cultures were incubated at 37°C under saturated humidity, 8% O₂ and 5% CO₂, in 4 well culture plates (Nunc, Denmark). The oocytes were stripped 16 to 20 hr after insemination. The presence of pronuclei was regarded as fertilisation in progress. Further culture was under identical conditions except that the serum concentration was raised to 15%.

The motility in general and the percentage of linearly progressing spermatozoa in each culture was recorded after oocyte stripping. The time of recording was approximately 24 hr after donation of the ejaculate. A control culture containing $10^5$ sperms/ml without oocyte was maintained and motility accordingly recorded. A second recording of motility was on the second day (about 48 hr). Prior to the IVF trials, in all cases sperm motility in culture had been recorded on a daily basis until extinction.

Data from 590 trials completed in this manner were compared for the frequency of fertilisation as a function of sperm motility. Statistical tests for significance by simultaneous comparisons of mean values was done by employing the multiple t-test according to Bonferroni.

The results are illustrated in the duplex histogram (Fig. 1) in which the ordinate gives the frequency (percentage) of either fertilisation or mean sperm motility recorded at 24 and 48 hr, respectively, in culture. The abscissa indicates the categorised mean sperm motility as obtained prior to the IVF trials. The anterior partially hatched columns give the recorded fraction of mean sperm motility in the IVF cultures at 24 hr (total column height) and at 48 hr (non-hatched part of the columns). The heights of the columns (for both 24 and 48 hr) reveal a gradient of about 45° which is an indication of absolute correlation between the motility estimates prior to IVF and during IVF. The posterior filled columns indicate the mean frequency of fertilisation as observed at the pronucleus stage. The numbers given in parentheses are the numbers of trials for each category.

Fig 1—Duplex histogram indicates the frequency in per cent (ordinate) for both mean sperm linear motility determined during oocyte stripping (total height of the anterior partially hatched columns) at 24 hr, and fertilisation as judged by the formation of pronuclei (dark columns). The abscissa indicates the linear motility of the same participants rounded off to the next higher decade as assessed in tests prior to IVF. The unshaded part of the anterior columns give the linear motility at IVF 48 hr later. The numbers in parentheses are the number of trials for the respective groups on the abscissa. The difference in fertilisation rates at 50% and 60% sperm motility is statistically highly significant ($P = 0.01$). The rates plateau thereafter.
Interestingly, no fertilisations were observed when sperm motility was below 50% at 24 hr in vitro. At 50% motility, the frequency of fertilisation was about 25%. At motilities of about 60% and more, fertilisation was around 70 to 75% which is the achievable maximum. The results suggest a threshold effect. The threshold would be the minimal motility at 24 hr required to initiate fertilisation. The maximal fertilising potential is acquired at motilities of 60% and higher. This may be regarded as a vitality index.

Currently available tests which aim at assessing the fertilising potential of human sperms have each some drawback or other. Conventional spermograms done in accordance with WHO recommendations have only restricted application in assisted reproduction, since all forms of assisted reproduction employ methods of sperm selection. Predictive assays ought, therefore, to be confined to the selected fraction in order to be relevant. Negligence of this aspect leads to prognostic failures. Apart from practical difficulties in making accurate visual notations of motilities in native semen specimens, these estimations could also be spurious because of variability in viscosity and osmolality of seminal plasma, both of which affect motility.

The Kremer Test has been useful for the estimation of sperm vitality. Its reliability depends on the viscosity of patients' cervical mucus which, however, is variable. Another drawback of this test which probably contributed to its lack of popularity is its tediousness. The use of standardised bovine mucus purported elimination of the major shortcoming of the Kremer Test. Some IVF centres try to use the observed motility after Swim Up as a predictive indicator. As mentioned above, although sperm motility may be taken as an index of vitality, it is temporally variable. Currently available tests, conceived to determine sperm vitality, do not estimate sperm asthena which is the inverse of vitality. This can be assessed by motility recordings from the time the sperms have been obtained until they cease to be motile.

Maintaining the sperms in culture medium under the same conditions used in IVF improves the reliability, besides facilitating easy microscopy in the culture plates. Motile sperms from the majority of men may be observed until day 4 to 5 in culture and in rare cases as long as 8 days. The other extreme is when sperms cease to show signs of motility within 24 hours. A scale of motility grades reflecting vitality or the inverse, asthena, can therefore be ascertained. The lower limit for initiating fertilisation is 50% linear motility at 24hr.

The appearance of a threshold is a decisive factor which enables the method to be deemed as an assay. The reliability of the observed threshold effect is, therefore, of critical importance. Whether the threshold is followed by a steep transition to a plateau cannot be established with certainty from the available data. The total height of the partially hatched columns (measure of sperm motility at IVF) stands in linear correlation with the prior estimated maximal motility values (on the abscissa), which is an indication of precision in the assay system.

As male subfertility owing to asthenospermia is often the cause of sterility and to date no cure is available, there is urgent need for a simple cost-effective predictive assay to determine the fertilising potential of sperms before counselling and further steps. Although ICSI may offer a solution, it is not a general practice in assisted reproduction, besides bioethical reservations. The assay presented here is simple and highly cost-effective without compromising reliability.

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References