Sperm morphology is not the only criterion of male infertility

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We agree with previous contributors to this debate that the goal of correctly estimating a man's fertility potential has been of great interest to many researchers for a long time (Siebel and Zilberstein, 1995; Aitken *et al.*, 1995; Barratt, 1995). Male fertility requires the timely deposition of spermatozoa in the the woman's vagina, adequate motility for spermatozoa to reach the egg, and normal fertilization capacity of spermatozoa. Beyond the information derived from a man's medical history and physical examination, semen analysis provides important indicators that predict fertility potential. The general relationship between the normal parameters of fresh semen and the expectation of conception *in vivo* has been acknowledged, and some characteristics have proved useful in distinguishing between fertile and infertile samples. Even though these parameters have been utilized for many years, the semen profile is a relatively blunt instrument for the diagnosis of the infertile male, even when carefully performed. Wallace *et al.* (1992) obtained conceptions in couples in which the men had sperm counts suppressed well into the infertile range, as a result of exogenous steroid therapy. However, the advent of in-vitro fertilization (IVF) has provided direct means of evaluating the fertility potential of a semen sample without interference from other infertility factors. In addition, IVF has become an important treatment option for male infertility, although fertilization rates are often lower than those for normospermic males, resulting in fewer embryos for transfer and, consequently, a lowered expectation of conception. Several studies have reported a correlation between fertilization rate and semen variables (sperm concentration, motility and morphology) in fresh samples or motile sperm fractions. Observations by Kruger *et al.* (1987, 1988) suggest that evaluation of sperm morphology by strict criteria is the best parameter to predict the sperm fertilizing ability in the in-vitro system. Since the pioneering study of MacLeod (1955), seminal cytology has been recognised as a source of clinically meaningful information. For many years MacLeod's morphological criteria have been accepted by the World Health Organisation (WHO, 1992) as the standard reference. By strict criteria, the fraction of normal forms in semen requires estimation not only as far as length, width and shape of the sperm head are concerned, but also according to the spatial distribution of the acrosome covering the head, as well as retention of any extra cytoplasm around the nucleus and midpiece. Other laboratories have developed different criteria for evaluating sperm morphological characteristics, but they have come to similar conclusions (Hinting *et al.*, 1990; Enginsu, 1991). According to these criteria, fertile men have >14% normal forms in their semen and men with ≤4% normal forms are generally regarded as subfertile (Grow *et al.*, 1994). In order to make this evaluation objective, several laboratories (Davis *et al.*, 1992; Kruger *et al.*, 1993) have recently developed computer programs to estimate sperm head shape characteristics as accurately and precisely as possible.

However, there are studies that criticize the importance of morphology in order to identify the subfertile male. Check *et al.* (1992a) reported that when motile spermatozoa densities were normally represented ($>10\times10^6$/ml), 87% of the female partners of men with normal sperm morphology (assessed according to WHO criteria) achieved pregnancies within 6 months, compared with 82% of couples with subnormal sperm morphology in the male partner. These authors suggest that the morphological parameters used for the WHO criteria are inadequate to distinguish normal from subnormal samples. Check *et al.* (1992a) also performed prospective and retrospective studies to evaluate sperm morphology using strict criteria for predicting fertilization capacity in males. The prospective study showed a 41% pregnancy rate in the ≤4% normal morphology group compared with a 29% rate in the >14% group (difference not significant). Retrospective analysis showed a 50% pregnancy rate in the group with ≤4% normal morphology score compared with a 67% pregnancy rate in the group with >14% (difference not significant). Technical experience is the essential requirement for a correct performance of sperm analysis and the comparison of results is difficult due to variations between different laboratories.

Some studies highlight how the method used to evaluate morphology is crucial in order to obtain comparable results. Meschede *et al.* (1993) used 158 random semen samples to analyse how the results of sperm morphology assessment were influenced by different techniques for preparing the slide for microscope assessment. The three techniques compared were the Papanicolau (PAP) stain, the Shor stain (SHO) and the wet preparation protocol (WET), which are currently recommended by WHO for use in andrology laboratories. Meschede *et al.* (1993) found a poor correlation between evaluation of PAP, SHO and WET slides and concluded that only one standard method should be recommended in order to ensure inter-laboratory comparability of results and to enhance the reliability of sperm morphology analysis for predicting fertility. Other investigators (Bartoov *et al.*, 1993) found that normal morphology does not seem to be sufficiently reliable as a sole predictor of male fertility potential and, using a multivariate discriminant analysis, reported that the combination of semen volume, sperm count, percentage of motility and normal forms, when considered in correct proportion, has more predictive value. Therefore, it is difficult to draw final conclusions on the basis of these published results.

Duncan *et al.* (1993) demonstrated a strong correlation between the fertilization rate and the combined indices of percentage of normal morphology and grade of motility in the fresh semen and percentage of progressive motility in the motile sperm fraction. The accuracy of prediction was 77% for poor fertilization (<35%) and 95% for acceptable (>35%) fertilization.

Enginsu *et al.* (1991, 1993) studied a possible correlation

between morphological evaluation of spermatozoa, using strict criteria and conventional sperm parameters, as far as IVF and pregnancy outcome before and after swim-up selection procedure are concerned. This study assessed the influence of the total number of spermatozoa and of the percentage of those with strictly normal morphology in the insemination sample. Results showed that the percentage of spermatozoa with normal morphology using strict criteria, both in fresh and in post swim-up samples, were the best predictors of IVF outcome. Their respective cut-off parameters were 5 and 8%. The number of morphologically normal spermatozoa utilized for insemination also showed a good correlation with fertilization percentages. If both parameters were below the cut-off points of 5% and 3×10^6/ml respectively, the fertilization rate per oocyte was very low and no pregnancy was achieved, while when both parameters were above the cut-off points, the fertilization rate per oocyte was 72% and the pregnancy rate per transfer was 27%.

Thanks to these studies, we may conclude that the assessment of sperm morphology is very important to predict results of IVF. However, in our opinion, the use of sperm morphology for predicting fertility is still insufficient. Other factors, such as total motile count before and after treatment, should also be considered. It is evident, in fact, that the value of morphology is different according to the variation in the number of spermatozoa in the sample. When the concentration of spermatozoa is very low, morphological assessment is more complex to perform and, therefore, less predictive. Moreover, we think that fertilization results may be very different, on the basis of the number of normal spermatozoa we use. Many investigators recommend an increased sperm concentration in the insemination medium in cases of sperm morphology of <14% normal forms. In this regard, Franken *et al.* (1990) evaluated the zona binding capacity of patients with abnormal sperm morphology, using standard hemizona assay conditions and increasing the sperm concentration for insemination. Using spermatozoa from teratozoospermic patients versus proven fertile controls, they found that the concentration of motile spermatozoa from teratozoospermic patients had to be significantly enhanced in order to obtain the same hemizona binding. We confirm the importance of examining all parameters; only by their correlation will it be possible to identify the group of patients with very low chances of success and for whom micromanipulation techniques may be indicated. In particular, intracytoplasmic sperm injection (ICSI) gives high fertilization rates in cases of severe oligoasthenoteratozoospermia (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1991, 1993). It is very important to define as precisely as possible the cut-off points, in order to avoid inappropriate use of IVF when chances are too low as well as avoiding the inappropriate use of ICSI, which is much more expensive for the couple. The indications for the use of micromanipulation methods are vague and can vary greatly from centre to centre. Indications such as male factor infertility or insufficient recovery of spermatozoa after preparation may merely reflect an inadequate sperm-processing methodology. Likewise, indications such as unacceptable for IVF or poor prognosis for IVF or previous failure of fertilization may not necessarily require micromanipulation in other hands. Therefore, it becomes very important to choose the right kind of treatment and culture conditions, as well as a correct evaluation of seminal parameters. The method for the separation of motile spermatozoa might be important in influencing the results. Many groups currently use two methods: swim-up and centrifugation on discontinuous Percoll gradient. Van Der Zwalmen *et al.* (1991) analysed the respective effects of swim-up and Percoll on sperm morphology in different types of ejaculates: 62 semen samples with normal parameters and 41 with poor parameters. Both separation techniques resulted in improved morphology in the final preparation but only the increase of morphologically normal spermatozoa in the final Percoll suspension was significant. Together with the improvement of sperm morphology, a higher pregnancy rate was obtained after Percoll. Ord *et al.* (1993) demonstrated the validity of IVF in severe male factor infertility. In this study, 71 couples with a total motility count of $<5\times10^6$ and <30% normal forms in the pretreatment sample were considered. A significant difference in fertilization rate was found when the total motility count was $<1.5\times10^6$ (54 versus 25% respectively, $P < 0.0001$).

Some consideration must be given to this study. Firstly, the authors pointed out the importance of the methodology used to treat samples with severe abnormality (use of Mini-Percoll, higher number of spermatozoa for insemination, type of culture system, adding the oocytes to the prepared spermatozoa and not *vice versa*). Secondly, it was possible to identify the group of patients in which results were likely to be significantly lower and ICSI was indicated.

Similarly, in our department, when we suspect a fertilization failure, we adopt different techniques in order to obtain an adequate sperm recruitment and to improve the possibility of spermatozoon–oocyte interaction. We can remove cumulus cells, we can use sperm stimulators and we can adopt micromanipulation techniques in microdroplets (50 µl) with oil-cultivated spermatozoa. Also, the means to improve fertilization could include the collection of multiple ejaculated samples or increase in sperm concentration near the oocyte.

In conclusion, we believe that the aim should be to find the minimum sperm parameters associated with specific methods of seminal treatment and with culture techniques which can lead to a single, common strategy for male infertility problems.

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