

ORIGINAL ARTICLE

Lack of Impact of Semen Quality on Fertilization in Assisted Conception

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Abstract

Background

Defective semen quality is one of the commonest causes of infertility. The diagnosis of male fertility depends upon a descriptive evaluation of human semen, however a normal semen analysis does not necessarily indicate satisfactory fertility potential.

Aims

(i) to examine the semen quality of patients undergoing treatment by assisted conception, (ii) to explore relationships between semen quality and treatment outcomes, and (iii) to look at inter-laboratory variation in the assessment of semen quality.

Methods

Semen quality in patients undergoing assisted conception treatment between 2001 and 2004 was reviewed. Data on female age, egg numbers and fertilization outcomes was obtained by case note review.

Results

The thresholds used to direct patients towards IVF or ICSI treatment were comparable with the normal values promulgated by WHO, with the exception of morphology. Semen quality was not predictive of fertilization rates. When the results of independent measurements of the same sample were compared, there was diagnostic disagreement in between 10% - 29% of samples.

Conclusions

The conventional criteria of semen quality are used to determine treatment strategy for couples undergoing assisted conception but are not reflected in fertilization rates, emphasising the limited utility of the conventional criteria of semen quality in the assessment of sperm function. There remains significant inter-laboratory variation in the results of semen analysis.

Key words

Semen quality, assisted conception, fertilization rates

Introduction

Infertility is an extremely common problem affecting between one in four and one in seven couples and has major public health, economic, and psychosocial consequences.^{1,2} The commonest single cause is defects in semen quality, with male problems being identified in over 40% of couples.³ Semen analysis, undertaken according to recognised international standards⁴ remains the most important laboratory investigation for men when assessing the infertile couple, although it is clear that there are significant variations between laboratories in the performance of this basic investigation.^{5,6} Since their development in the late 1970s, techniques of assisted conception have come to play a major role in the treatment of infertile couples, and in some European countries, as many as 4% of children are now conceived in-vitro.⁷ Advances in in-vitro fertilization (IVF) techniques, particularly intra-cytoplasmic sperm injection (ICSI) involving the direct injection of a single spermatozoon into an egg, have revolutionised the management of couples with male factor subfertility, but treatment choices are based upon a conventional semen profile. This study sets out to examine the semen quality of patients undergoing treatment by assisted conception in one centre, to explore relationships between semen quality and treatment outcomes, and lastly to look at inter-laboratory variation in the assessment of semen quality.

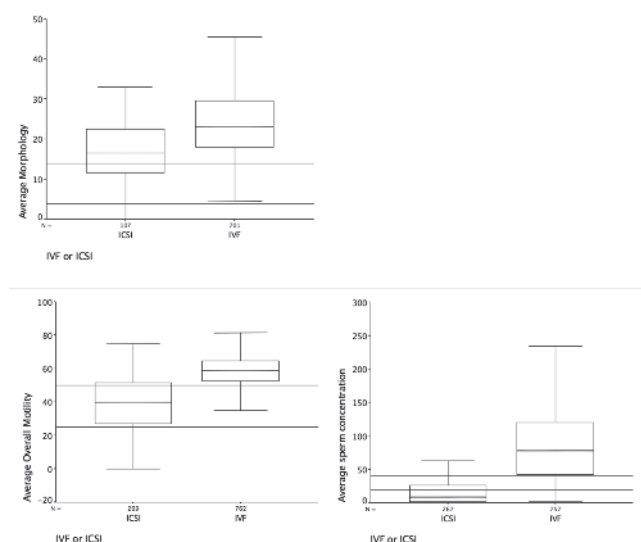
Materials and methods

Semen samples were obtained from the male partners of 731 successive couples undergoing 1031 assisted reproductive treatment cycles between 2001 and 2004 at the Edinburgh Fertility and Reproductive Endocrine Centre. Of these, 762 treatment cycles (74%) were IVF and 269 cycles (26%) were ICSI. The semen samples were collected at the centre on the day of oocyte retrieval after a recommended two - three days of sexual abstinence. After liquefaction, the spermatozoa were analyzed by two separate laboratories, both according to WHO criteria;⁴ the IVF laboratory using a Makler chamber⁸ and the andrology laboratory using a Neubauer haemocytometer. The semen quality data was collected and entered into an excel database, while data on female age, egg numbers and fertilization outcomes was obtained by case note review. The data were analysed using SPSS. In comparing treatment outcomes with semen quality the average value of the determinations made by the two laboratories was used.

Results

1. Measures of semen quality: About 50% of treatments occurred in women aged over 35 years. The average number of oocytes retrieved was comparable between IVF and ICSI cycles, in IVF cycles seven and in ICSI, eight. The median fertilization rate was also similar, being 60% in IVF and 70% in ICSI. Seventy-five percent of IVF treatment cases had a sperm concentration over 40 x 10⁶/ml and 66% of ICSI treatment cases had a sperm concentration less than 20 x 10⁶/ml. Three quarters of IVF treatment cases had overall motility of sperm above 50% and one quarter of all ICSI patients had overall motility of sperm below 25%. It has been suggested that, as sperm morphology falls below 15% normal forms, the fertilization rate in-vitro decreases.⁹ The median morphology for IVF patients was around 25% and median morphology for ICSI patients was around 18% (Figure 1).

Figure 1: Semen Quality in couples undergoing IVF and ICSI treatment, showing recognised values for normality.



2. Impact on fertility: To look at outcomes, we examined treatment cycles where more than four oocytes were collected. We compared the fertilization rates with average sperm concentration, average overall motility and average morphology in both IVF and ICSI treatment cycles using multiple linear regression and multivariate discriminate analysis. Poor fertilization was defined as less than 50% of oocytes fertilized. There was no correlation between sperm concentration, motility and morphology to the fertilization rate in these analyses. There were 27 cases (4%) with failed fertilization in the IVF group even when average sperm concentration was more than the WHO threshold of 20 million/ml. Failed fertilization was very rare in ICSI treatment (Figure 2 and Table I).

3. Comparison between the two laboratories: Table II shows the comparison of sperm concentration by both IVF and Andrology laboratories. Both laboratories confirmed normozoospermic samples in 729 (71.2%) cases and oligozoospermic samples in 201 (19.6%) cases. However there was diagnostic discordance in assessment of sperm concentration in 94 (9.2%) of cases. When comparing the progressive motility, there was agreement on normozoospermic samples in 689 (67%) cases and asthenozoospermic samples

Figure 2: Semen Quality and Fertilization Rates
Top panel = IVF, Bottom panel = ICSI.

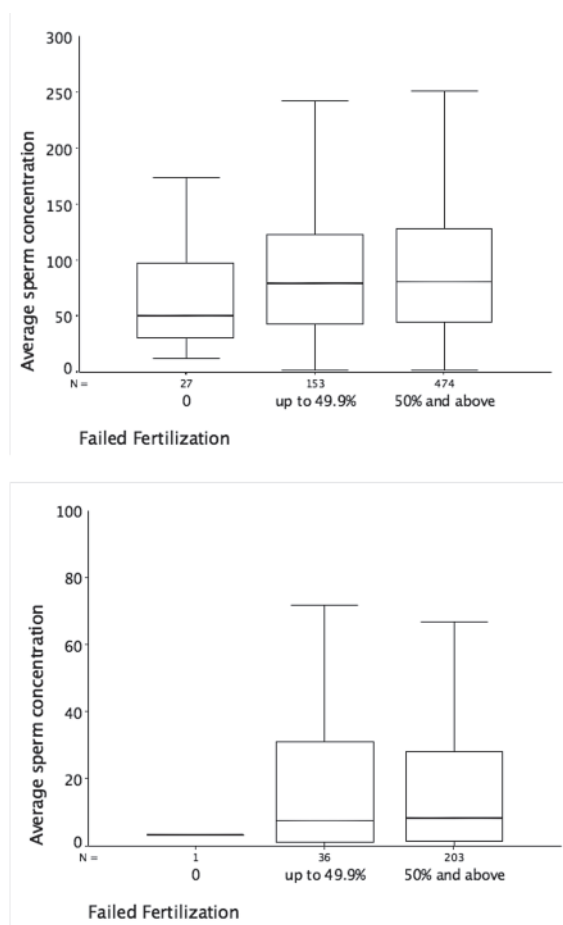


Table I: Fertilization Rate in cycles with ≥ 4 eggs inseminated (% of cases)

	All treatment cycles	ICSI	IVF
No of cycles (=100%)	899	242	657
Failed Fertilization (%)	3.1	0.4	4.1
Up to 49% Fertilization	21.0	14.9	23.3
50 - 100% Fertilization	75.9	84.7	72.6

Table II: Agreement between laboratories in the diagnosis of Oligozoospermia (<20 x 10⁶/ml)

	Diagnosis by laboratory 2	
Diagnosis by laboratory 1	Oligozoospermic	Normozoospermic
Oligozoospermic	201 (19.6%)	47 (4.6%)
Normozoospermic	47 (4.6%)	729 (71.2%)

in 119 (11.6%) cases and there is diagnostic discordance in assessment of progressive motility in 221 (21.5%) of cases (Table III). Using normal morphology below 14% as the definition of teratozoospermia, there was diagnostic discordance in assessment of normal morphology in 232 (28.7%) of cases (Table IV).

Table III: Agreement between laboratories in the diagnosis of Asthenozoospermia (progressive motility <25%)

Diagnosis by laboratory 1	Diagnosis by laboratory 2	
	Asthenozoospermic	Normozoospermic
Asthenozoospermic	119 (11.6%)	200 (19.4%)
Normozoospermic	21 (2.0%)	689 (67.0%)

Table IV: Agreement between laboratories in the diagnosis of teratozoospermia (normal morphology <14%)

Diagnosis by laboratory 1	Diagnosis by laboratory 2	
	Teratozoospermic	Normozoospermic
Teratozoospermic	34 (4.2%)	151 (18.7%)
Normozoospermic	81 (10.0%)	542 (67.1%)

Discussion

Semen analysis is considered as a cornerstone of the laboratory evaluation of the infertile male and helps to define the severity of male factor infertility. However, our study serves to underline the comparatively limited utility of the conventional criteria of semen quality in the prediction of sperm function.¹⁰ It is notable, therefore, that the reference values promulgated by WHO are not strongly predictive of the achievement of pregnancy by infertile couples.¹⁰ Amongst fertile couples, it has been noted that as sperm concentrations rise, so the monthly chance of pregnancy rises, reaching a maximal value at sperm concentrations of 40 - 50 x 10⁶/ml¹¹, a figure which is very different from the commonly used value of 20 x 10⁶/ml. Nevertheless it is clear from the present study that the conventional criteria of semen quality, and the reference values promulgated by WHO, are being used in clinical practice to make very significant clinical decisions, and to select appropriate treatment options for couples undergoing assisted conception treatment. It is, thereafter, perhaps not surprising that in this analysis there was no clear relationship between sperm concentration, sperm motility and normal morphology and fertilization rates.

Several studies have looked at the relationships between attributes of semen quality and the outcomes of assisted conception. Nallella et al¹² have suggested that sperm motility and concentration may provide more accurate information than morphology during infertility evaluation and Grunert et al¹³ noted that while sperm concentration did not correlate strongly with fertilization rate, sperm motility and morphology were the most meaningful parameters in predicting fertilization success, a drop in fertilization rate being found when sperm motility or normal

morphology were below 40%. It has also been suggested that total motile sperm count, particularly when assessed before the start of IVF cycle and at the time of ovum pick may allow an accurate prediction of the chance of total fertilization failure.¹⁴ However, overall, the literature is inconclusive on the use of the conventional criteria of semen quality in the context of assisted conception treatment.

Moving beyond the conventional criteria of semen quality, several studies have looked at more detailed attributes of sperm function in relation to the prediction of IVF outcomes. But again, on reviewing the literature, it is striking that no consensus has emerged regarding which tests might be used, or how helpful they might be. Sukcharoen et al¹⁵ found that the fertilizing ability of human spermatozoa was related to sperm morphology, attributes of sperm movement and reactive oxygen species production and also suggested that the time delay between testing and IVF did not appear to affect predictive accuracy. Similarly Jedrzejczak et al¹⁶ showed that the best predictive factors for oocyte fertilization were normal morphology of sperm before and after density gradient selection, attributes of sperm movement in semen, and DNA content after density gradient centrifugation, which together accounted for 76.7% of fertilization prediction. Mahutte et al¹⁷ suggested that strict sperm morphology assessment, sperm-zona binding ratios and zona pellucida induced acrosome reaction tests may provide improved ability to predict failed fertilization. A range of in-vitro tests have been developed to monitor various aspects of sperm function including their potential for movement, cervical mucus penetration, capacitation, zona recognition, the acrosome reaction and sperm-oocyte fusion. Such functional tests have been found to predict the fertilizing capacity of human spermatozoa in-vitro and in-vivo with some accuracy. Recent developments in this field have included the introduction of tests to assess the degree to which spermatozoa have suffered oxidative stress as well as the integrity of their nuclear and mitochondrial DNA. Such assessments not only yield information on the fertilizing capacity of human spermatozoa but also their ability to support normal embryonic development.¹⁸

The most recent novel approach to predicting outcomes is sperm DNA integrity assessment. The sperm chromatin structure assay has been suggested to be a useful tool in diagnosis and may contribute to the prognosis for the fertility outcome of conventional IVF but to a lesser extent in the ICSI procedure.¹⁹ Irvine et al²⁰ have noted highly significant negative correlations between DNA fragmentation and semen quality, particularly sperm concentration. Multiple regression analysis indicated that other attributes of semen quality such as sperm movement and ROS generation, were also related to DNA damage. They concluded that a significant proportion of infertile men have elevated levels of DNA damage in their ejaculated spermatozoa. However, although several studies have suggested that such tests may have predictive value for IVF outcomes, over and above the conventional criteria of semen quality, a recent review of the literature has suggested that this predictive value is of limited clinical utility.²¹ The available tests of sperm function are complex and time-consuming, and for the moment have limited applicability out with the context of properly designed research studies.

In spite of the important clinical decisions being based upon semen analysis data, it is clear that there is a significant lack of standardization in the performance and reporting of semen analyses among laboratories.^{5,6} Quality control procedures have only recently been implemented widely in andrology laboratories.²² Both of the laboratories involved in the present

study were involved in external quality assurance schemes, and both had systems of internal quality control in place. Nevertheless, our study showed significant inter-laboratory variation in the results of semen analysis. Pacey et al²³ have stressed the need for laboratories to participate in internal and external quality assurance activities, incorporate rigorous training protocols for technical staff and use reliable procedures for total quality management. It remains to be seen what impact these developments will have on the utility of and diagnostic value of this test.

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