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Predictive value of normal sperm morphology: a structured literature review

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The aim of the study was to conduct a structured review of the literature published on the use of normal sperm morphology, as an indicator of male fertility potential in the in-vitro fertilization (IVF) situation, and to establish the universal predictive value of this semen parameter. Published literature in which normal sperm morphology was used to predict fertilization and pregnancy, during the period 1978–1996, was reviewed. A total of 216 articles were identified by the sourcing methodology, but only 49 provided data that could be tabulated and analysed. Of these, only 18 provided sufficient data for statistical analysis. Fifteen studies used the strict criteria to evaluate sperm morphology, two used World Health Organization (WHO) guidelines and one used both the strict criteria and the WHO guidelines. All the studies (n = 10) using the 5 and 14% normal sperm morphology thresholds (strict criteria) produced positive predictive values for IVF success. In the prediction of pregnancy, 82% (9/11) and 75% (6/8) of the studies produced positive predictive values when using the 5% and 14% thresholds respectively. Aggregating the data produced around the 5% normal sperm morphology threshold (strict criteria), the overall fertilization rates were 59.3% (1979/3337; per oocyte) for the ≤4% group and 77.6% (10345/13327; per oocyte) for the >4% group, and the overall pregnancy rates were 15.2% (60/395; per cycle) and 26.0% (355/1368; per cycle) respectively. The no-transfer rates across the 5% threshold were 24.0% (86/359; per cycle) in the ≤4% group compared to 7.4% (80/1088; per cycle) in the >4% group. The inclusion of an accurately evaluated normal sperm morphology count as an integral part of the standard semen analysis makes this analysis still the most cost-effective means of evaluating the male factor.

Key words: human/in-vitro fertilization/normal sperm morphology/pregnancy/structured review

Introduction

With the realization that male fertility was an important contributor to the conception potential of a couple, establishment of the fertility potential of men became a subject of intense research. This included the close examination (light microscopy, computerized analysis etc.) of the conventional sperm parameters (morphology, motility, forward progression and concentration) (Liu *et al*., 1990; Wang *et al*., 1991; Enginsu *et al*., 1992a,b) and the use of functional assays (Coetzee *et al*., 1989; Franken *et al*., 1989; Chan *et al*., 1990; Henkel *et al*., 1993) to distinguish between fertile and infertile men and correlate these parameters and outcomes with in-vivo conception and in-vitro fertilization (IVF), implantation and pregnancy. Understandably, no single test or sperm parameter was found to be absolute in its prediction of male fertility or infertility, as no single sperm feature or function could truly represent the ability of spermatozoa to accomplish the complex sequence of events leading to a clinical pregnancy.

IVF provides the best means of examining sperm–egg interaction and determining fertilization probability for diagnostic purposes, but obviously cannot be used as a routine screening assay. Therefore, due to the cost, time and ethical constraints of IVF and functional assays, the correct evaluation of the basic semen parameters still remains the most cost-effective diagnostic tool for male fertility.

Even though the basic semen parameters are descriptive in nature, several studies have obtained good correlations between IVF and motility (Alper *et al.*, 1985; Ron-El *et al.*,

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1991; Robinson *et al.*, 1994), concentration (Biljan *et al.*, 1994; Calvo *et al.*, 1994; Robinson *et al.*, 1994) and normal/abnormal sperm morphology (Kruger *et al.*, 1986; Enginsu *et al.*, 1992a; Grow *et al.*, 1994). Of all the semen parameters, sperm morphology has consistently been the best indicator of male fertility. Many authors have gone as far as to argue that sperm morphology is a reflection of sperm functional competence. The main shortcomings of this parameter have been the large number of classification systems used to describe what factors constitute a morphologically normal/abnormal spermatozoon, the various staining procedures employed and the subjective nature of the evaluation. The following are some of the major classification systems used to classify normality: Eliasson (1971), World Health Organization (WHO; 1980, 1987, 1992), Williams (1964), Tygerberg strict criteria (Kruger *et al.*, 1986, 1988; Menkveld *et al.*, 1990), David *et al.* (1975), Freund (1966), Fredricsson (1979) and Düsseldorf (Hofmann *et al.*, 1985).

The aim of this study was to establish the universal predictive value of normal sperm morphology, in the IVF situation, by means of a structured literature review.

Materials and methods

The articles included in the review were primarily found by means of a computerized Medline search using specific criteria (key words: IVF, pregnancy and normal sperm morphology; limitations: English, human and within the period 1978–1996). Our unit's data bank of articles was also searched using the same criteria; finally, the references of the articles obtained were cross-checked. Articles were only analysed further if certain criteria were met: (i) statistical associations were investigated between sperm morphology and IVF and/or pregnancy, (ii) abnormal/normal sperm morphology fertility thresholds were identified and (iii) whether descriptive data (per oocyte fertilization, per cycle/transfer pregnancy rates and pregnancy outcome) were presented.

A total of 216 articles were identified by the initial search, of which only 49 satisfied more than one of the above selection criteria. The 49 selected articles are chronologically listed in Table I. These selected articles were independently analysed by two of the authors (K.C. and T.F.K.) and the results tabulated by consensus.

Table I. Studies that analysed the association between seminal parameters and in-vitro outcomes

Authors	Classification	Stain method	Best predictor(s)/best classification
Mahadevan & Trouson (1984)	Eliasson (1971)	Eosin yellow	Abnormal sperm forms and motility
Yovich & Stanger (1984)	WHO (1980)	Not given	35 y 10^6 motile spermatozoa/ml
Alper *et al.* (1985)	WHO (1980)	Formalin and haematoxylin	Sperm count and motility
Hirsh *et al.* (1986)	WHO (1980)	Not given	Sperm density and motility
Jeulin *et al.* (1986)	David *et al.* (1975)	Shorr	Acrosomal morphology and amplitude of lateral head displacement
Jeyendran *et al.* (1986a)	Williams (1964)	Papanicolaou	Normal acrosome
Jeyendran *et al.* (1986b)*	mWilliams & WHO (1980)	Papanicolaou	mWilliams
Kruger *et al.* (1986)	Strict criteria	Papanicolaou	Normal sperm morphology
Kruger *et al.* (1987)	Strict criteria	Diff-Quik	Normal sperm morphology
Talbert *et al.* (1987)	Freund & Petersen (1976)	Not given	Forward progression and white blood cell count
Comhaire *et al.* (1988)	WHO (1987)	Papanicolaou	Normal sperm morphology and progressive motility
Kruger *et al.* (1988)	Strict criteria	Diff-Quik	Normal sperm morphology
Liu & Baker (1988)	WHO (1980)	Shorr	Normal sperm morphology and insemination concentration
Liu *et al.* (1988)	WHO (1980)	Shorr	Insemination count, normal sperm morphology and vitality
Oehninger *et al.* (1988)	Strict criteria	Diff-Quik	Normal sperm morphology
Chan *et al.* (1989)	WHO (1987)	Papanicolaou	Normal sperm morphology and computer-assisted sperm movement characteristics
Hinting *et al.* (1989)	WHO (1987)	Not given	Normal sperm morphology
Chan *et al.* (1990)	WHO (1987)	Not given	Normal sperm morphology insemination concentration and normal intact acrosomes

Table I. *Continued*

Authors	Classification	Stain method	Best predictor(s)/best classification
Rosenborg *et al.* (1990)	Fredericsson (1979)	Deoxycycline & metronidazole	Not given
Sevenster *et al.* (1990)	Strict criteria	Papanicolaou	Normal sperm morphology
Barlow *et al.* (1991)	WHO (1987)	Eosin–nigrosin	Normal sperm morphology and swim-up motility
Enginsu *et al.* (1991)*	Strict criteria & WHO (1987)	Diff-Quik	Strict criteria
Kobayashi *et al.* (1991)	Strict criteria	Diff-Quik	Normal sperm morphology
Ron-El *et al.* (1991)	Strict criteria	Eosin–nigrosin	Normal sperm morphology and motile spermatozoa
Coates *et al.* (1992)	Strict criteria	Not given	None of the semen parameters
De Geyter *et al.* (1992)	WHO (1987)	Papanicolaou	Normal sperm morphology and progressively motile spermatozoa
Enginsu *et al.* (1992a)	Strict criteria	Diff-Quik	Normal sperm morphology and progressive motile spermatozoa
Enginsu *et al.* (1992b)*	Strict criteria & WHO (1987)	Diff-Quik	Normal sperm morphology
Liu & Baker (1992)	WHO (1987)	Shorr	Normal sperm morphology
Duncan *et al.* (1993)	WHO (1987)	Papanicolaou	Normal sperm morphology and progressive motility in the insemination sample
Enginsu *et al.* (1993)	Strict criteria	Diff-Quik	Normal sperm morphology
Biljan *et al.* (1994)	WHO (1987)	Not given	Sperm concentration
Calvo *et al.* (1994)	Strict criteria	Diff-Quik	Normal sperm morphology and sperm concentration
Grow *et al.* (1994)	Strict criteria	Diff-Quik	Normal sperm morphology
Liu & Baker (1994a)	mWHO (1987)	Shorr	Normal sperm morphology
Ombelet *et al.* (1994)	Strict criteria	Papanicolaou	Normal sperm morphology
Robinson *et al.* (1994)	Strict criteria	Testsimplet	Sperm concentration and motility
Hofmann *et al.* (1995)*	Ideally normal, strict normal sperm and acrosomal morphology, strict criteria & Düsseldorf	Papanicolaou	Düsseldorf
Morgentaler *et al.* (1995)*	WHO (1992) & strict criteria	Not given	WHO (1992)
Sukcharoen *et al.* (1995)	wWHO (1992)	Wet preparation	Normal sperm morphology
Yue *et al.* (1995)	Strict criteria	Papanicolaou	Normal sperm morphology
Yang *et al.*,(1995)	Strict criteria	Papanicolaou	Normal sperm mrophology
Al-Hasani *et al.* (1996)	Strict criteria	Papanicolaou	Normal sperm morphology
Figueiredo *et al.* (1996)	Strict criteria	Papanicolaou	Normal sperm morphology
Harrison & Harrison (1996)*	WHO (1992) & strict criteria	Diff-Quik	Strict criteria
Hernandez *et al.* (1996)	Strict criteria	Haematoxylin & Brilliant Green	Normal sperm morphology
Menkveld *et al.* (1996)	Strict criteria	Papanicolaou	Normal sperm morphology and acrosomal index
Vawda *et al.* (1996)	Strict criteria	Papanicolaou, Spermac, Diff-Quik	Normal sperm morphology

m = modified; w = washed.
*Comparison of classification systems.

Where possible, odds ratio (OR) and 95% confidence interval (CI) analysis was performed on the number of oocytes fertilized and on the number of pregnancies obtained per cycle within certain normal sperm morphology thresholds (Lau and Chalmers, 1995). Pregnancy per cycle was chosen in preference to ongoing pregnancy rate, even

though the latter represents greater consensus in pregnancy definition, because a greater number of studies have published this figure. Studies with 0 counts (fertilization or pregnancy rate) were given the value of 0.5 to enable the estimation of OR. The studies included in the review were all observational and therefore no global estimates of the associations were made.

We do not contend that this review is complete, but only that the articles reviewed constitute a representative sample of studies published on the predictive value of sperm morphology in the IVF situation.

Results

Of the 49 articles analysed (Table I), 43 statistically compared the predictive value of a single sperm morphology classification system, while six articles statistically compared the predictive value of more than one normal sperm morphology classification system. The majority (81.4%; 35/43) of the articles concluded in their closing remarks that normal sperm morphology, including acrosomal morphology, had a role to play in the diagnosis of male fertility potential (Table II). Statistical analysis could, however, only be performed on 18 studies, due to the lack of adequate descriptive data (Tables III, IV and V).

Table II. The proportion of articles that obtained good (GPV) or poor prediction values (PPV), with regard to fertilization *in vitro*, using the different classification systems

Classification system	GPV	PPV	Total
WHO (1980)	2	3	5
WHO (1987)	10	1	11
WHO (1992)	1	0	1
Strict criteria	19	2	21
Other	3	2	5
Total	35	8	43

The largest proportion (48.8%;21/43) of the articles evaluated the association between the strict criteria normal morphology outcomes and fertilization and/or pregnancy (Table II); 90% (19/21) of these studies obtained a positive association with fertilization and/or pregnancy (Table II). Seventy six percent (13/17) of studies using the WHO classifications (1980, 1987, 1992) also obtained a useful association. In the six studies comparing the predictive value of different normal sperm morphology classification systems, three preferred the strict criteria, one a modified Williams (1964) classification, one the WHO (1992) criteria and one the Düsseldorf classification (Table I).

The articles (with data) statistically analysed for the predictive value of normal sperm morphology with regards

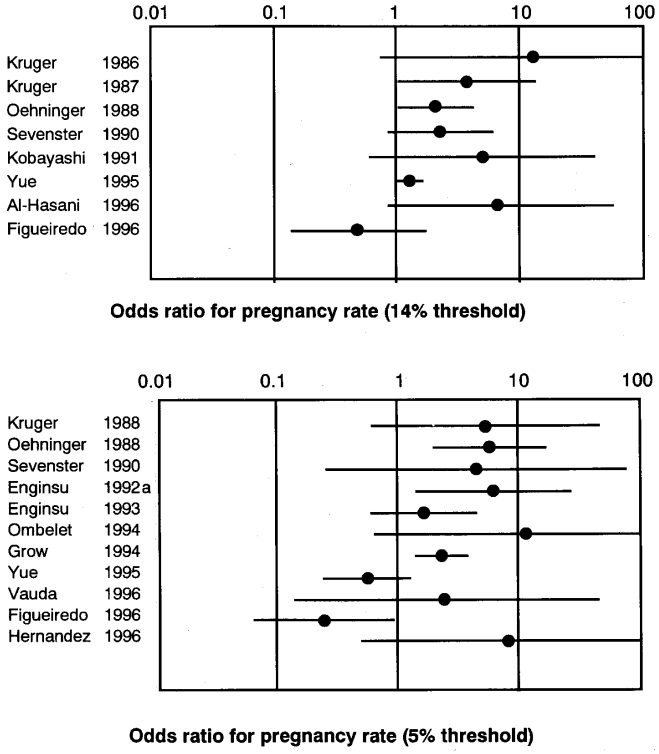

Figure 1. Odds ratios and confidence intervals for the predictive value of normal sperm morphology (strict criteria) for pregnancy rate per cycle.

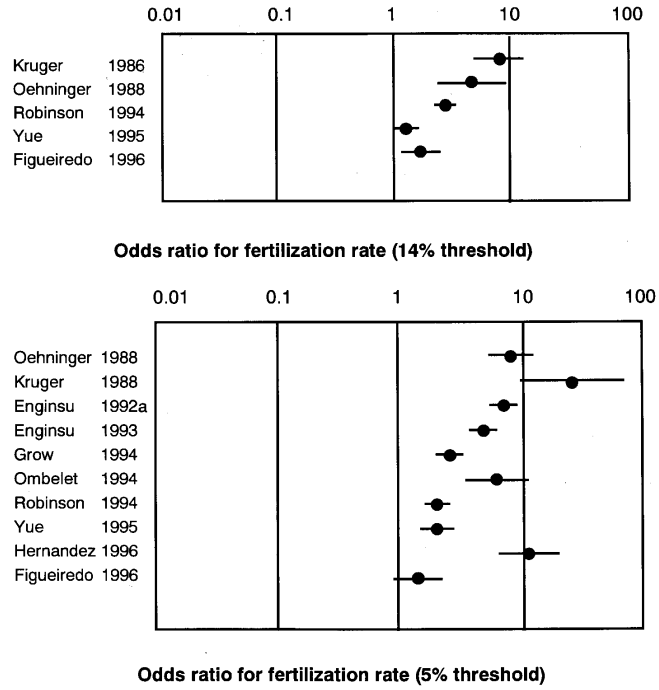

Figure 2. Odds ratios and confidence intervals for the predictive value of normal sperm morphology (strict criteria) for fertilization *in vitro*.

to fertilization and pregnancy form a very heterogeneous group, due to the different materials and methods used (i.e. stimulation protocols, sperm cell staining procedures, insemination concentrations, sperm preparation procedures, number of embryos transferred, embryo transfer technique, pregnancy validation, etc.). Fifteen of the 18 articles with data used the strict criteria as the sperm morphology classification system, while two used WHO (1980, 1992) guidelines and one used both the WHO and the strict criteria systems.

Using a 5% threshold (strict criteria), 10 studies provided data that could be analysed for the prediction of fertilization and 11 studies for the prediction of pregnancy (Table III). All the studies showed a positive predictive value for fertilization *in vitro*, with only one [Figueiredo *et al.*, 1996; OR = 1.42(CI: 0.90–2.25)] not reaching significance (Figure 2). In the prediction of pregnancy (per cycle), nine studies obtained a positive predictive value. The predictive value of the studies by Oehninger *et al* (1988), Enginsu *et al.* (1992a) and Grow *et al.* (1994) reached significance (Figure 1). Using a 14% threshold (strict criteria), five studies provided data that could be analysed for the prediction of fertilization and eight studies for the prediction of pregnancy (Table IV). Similar to the 5% analysis, all these studies showed positive and significant predictive value with regards to fertilization *in vitro* (Figure 2). In the prediction of pregnancy, two studies (Yue *et al.*, 1995; Figueiredo *et al.*, 1996) did not obtain a positive predictive value, while the studies of Oehninger *et al.* (1988) and Kruger *et al.* (1987) were both positive and significant (Figure 1).

The overall fertilization rates using the 5% normal sperm morphology threshold were 59.3% (1979/3337) for the ≤4% group and 77.6% (10 345/13 327) for the >4% group. The overall pregnancy rates around this threshold were 15.2% (60/395) for the ≤4% group compared to 26.0% (355/1368) for the >4% group. The overall fertilization rates using the 14% normal sperm morphology threshold were 72.7% (4511/6209) for the ≤14% group and 83.6% (2780/3325) for the >14% group. The overall pregnancy rates around this threshold were 24.3% (130/534) for the ≤14% group compared to 25.2% (164/651) for the >14% group.

Table III. Studies with data in which the 5% strict criteria threshold could be used to evaluate the predictive value (fertilization and pregnancy) of normal sperm morphology

Reference	<5%			≥5%			Odds ratio	
	n	FR (%)	P/C (%)	*n*	FR (%)	P/C (%)	Fertilization	Pregnancy/cycle
Oehninger *et al.* (1988)	47	47.0 (71/151)	8.5 (4/47)	185	87.7 (642/732)	35.7 (66/185)	8.04 (5.45–11.85)	5.96 (2.05–17.34)
Kruger *et al.* (1988)	13	7.9 (5/76)	7.7 (1/13)	32	63.8 (83/130)	31.2 (10/32)	25.08 (9.46–66.48)	5.45 (0.62–47.90)
Sevenster *et al.* (1990)	13	Not given	0.0 (0/13)	138	Not given	13.8 (19/138)		4.41 (0.25–77.16)
Enginsu *et al.* (1992a)	39	25.9 (105/406)	5.1 (2/39)	161	71.2 (1240/1741)	25.5 (41/161)	7.10 (5.55–9.07)	6.32 (1.46–27.39)
Enginsu *et al.* (1993)	33	32.3 (104/322)	15.2 (5/33)	152	69.6 (1103/1584)	23.0 (35/152)	4.81 (3.72–6.22)	1.68 (0.60–4.66)
Grow *et al.* (1994)	172	80.8 (1076/1332)	17.4	172 (30/172)	91.3	33.1	2.51 (1.99–3.17) (1224/1340)	2.35 (1.41–3.89) (57/172)
Ombelet *et al.* (1994)	12	37.9 (22/58)	0.0 (0/12)	88	79.1 (367/464)	30.7 (27/88)	6.19 (3.48–11.01)	11.18 (0.64–195.64)
Robinson *et al.* (1994)	86	68.7 (438/638)	Not given	724	81.3 (4329/5328)	Not given	1.98 (1.65–2.37)	
Yue *et al.* (1995)*	25	46.4 (77/166)	52.0 (13/25)	172	63.6 (744/116)	37.2 (64/172)	2.02 (1.46–2.81)	0.55 (0.24–1.27)
Hernandez *et al.* (1996)	17	22.6 (21/93)	0.0 (0/17)	95	76.7 (299/390)	18.9 (18/95)	11.27 (6.57–19.33)	8.35 (0.48–145.38)
Figueiredo *et al.* (1996)	14	62.1 (59/95)	35.7 (5/14)	63	69.9 (314/449)	11.1 (7/60)	1.42 (0.90–2.25)	0.24 (0.06–0.91)
Vawda *et al.* (1996)	10	Not given	0.0 (0/10)	110	Not given	10.0 (11/110)		2.43 (0.13–44.20)

95% confidence intervals; *n* = number of cycles; FR = fertilization rate (per oocyte); P/C = pregnancy per cycle rate.
*After Percoll preparation.

Table IV. Studies with data in which the 14% strict criteria threshold could be used to evaluate the predictive value (fertilization and pregnancy) of normal sperm morphology

Reference	≤14%			>14%			Odds ratio	
	n	FR (%)	P/C (%)	*n*	FR (%)	P/C (%)	Fertilization	Pregnancy/cycle
Kruger *et al.* (1986)	22	36.5 (38/104)	0.0 (0/22)	168	82.5 (579/702)	22.0 (37/168)	8.18 (5.24–12.75)	12.83 (0.76–216.557)
Kruger *et al.* (1987)	25	Not given	12.0 (3/25)	71	Not given	33.8 (24/71)		3.74 (1.02–13.78)
Oehninger *et al.* (1988)	191	77.9 (566/727)	27.2 (52/191)	41	94.2 (147/156)	43.9 (18/41)	4.65 (2.32–9.31)	2.09 (1.04–4.19)
Sevenster *et al.* (1990)	90	Not given	8.8 (8/90)	61	Not given	18.0 (11/61)		2.26 (0.85–5.99)
Kobayashi *et al.* (1991)[a]	13	Not given	7.7 (1/13)	110	Not given	29.1 (32/110)		4.92 (0.61–39.45)
Robinson *et al.* (1994)	556	75.9 (3233/4257)	Not given	254	89.8	Not given (1534/1709)	2.78 (2.34–3.30)	
Yue *et al.* (1995)[b]	132	59.7 (534/895)	43.9 (58/132)	65	65.2 (287/440)	29.2 (19/65)	1.27 (1.00–1.61)	0.53 (0.28–0.99)
Figueiredo *et al.* (1996)	33	61.9 (140/226)	21.2 (7/33)	44	73.3 (233/318)	11.4 (5/44)	1.68 (1.17–2.43)	0.48 (0.14–1.66)
Al-Hasani *et al.* (1996)[c]	28	Not given	3.6 (1/28)	91	Not given	19.8 (18/91)		6.66 (0.85–52.32)

95% confidence intervals; *n* = cycles; FR = fertilization rate (per oocyte); P/C = pregnancy per cycle rate.
[a]Actual cut-off 12%.
[b]After Percoll preparation.
[c]Actual cut-off 10%.

Table V. Studies with data in which 'other' criteria thresholds could be used to evaluate the predictive value (fertilization and pregnancy) of normal sperm morphology

Reference	FT	Infertile			Fertile			Odds ratio	
		n	FR (%)	P/C (%)	*n*	FR (%)	P/C (%)	Fertilization	Pregnancy/cycle
Mahadevan and Trouson (1984)	≥60%	66	58.7 (101/172)	4.5 (3/66)	292	73.5 (696/947)	13.7 (40/292)	1.95 (1.39–2.73)	3.33 (0.10–11.17)
Yovich and Stanger (1984)	≥60%	10	79.5 (31/39)	10.0 (1/10)	27	85.0 (51/60)	14.8 (4/27)	1.46 (0.51–4.19)	1.57 (0.15–15.97)
Yue *et al.* (1995)*	≥30%	76	57.1 (285/499)	52.6 (40/76)	121	64.1 (536/836)	30.6 (37/121)	1.34 (1.07–1.68)	0.40 (0.22–0.72)

95% confidence intervals; FT = fertility thresholds; *n* = cycles; FR = fertilization rate (per oocyte); P/C = pregnancy per cycle rate.
[a]After Percoll preparation.

Of the three studies (Table V) using 'other' (Eliasson, 1971; WHO, 1980) normal sperm morphology classification criteria, all produced positive outcomes with regards to fertilization *in vitro* and two with regards to pregnancy outcome. Two of the studies reached significance in the prediction of fertilization (Mahadevan and Trouson, 1984; Yue *et al.*, 1995), while none reached significance in the prediction of pregnancy.

Discussion

The debate on the role of normal sperm morphology in IVF has been continued by this article in the hope of promoting understanding of its value in the management of the infertile couple. To ensure that the basis of our arguments was unbiased we reviewed all the literature available on the subject for the period 1978–1996. The greatest disappointments of

this review were the low number (n = 18) of studies presenting their descriptive data for analysis [three studies using the WHO (1980, 1987, 1992) classification systems and 16 studies using the strict criteria] and the heterogeneity of the studies, which prevented the performance of a meta-analysis.

The simplicity of sperm morphology evaluation is simultaneously its greatest advantage and disadvantage. While it only requires standard laboratory equipment and between 10 min (Diff-Quik) and 2 h (Papanicolaou) processing time, it is difficult to perceive how something as simple and abstract as the form of a spermatozoon can represent its functional capacity, i.e. its ability to complete the complex sequence of events leading to normal fertilization and embryo development. Nevertheless, the majority of the studies reviewed (35/43; 81.4%) showed that the percentage of normal sperm morphology was positively associated with IVF outcome. This association was not restricted to any one particular classification system and/or evaluation procedure. Some of these studies also showed that this association was independent of any of the other semen parameters (Mahadevan and Trouson, 1984; Kruger *et al.*, 1986; Oehninger *et al.*, 1988; Liu and Baker, 1990; Grow *et al.*, 1994).

No study has, however, found normal sperm morphology to be absolute in its prediction, which is understandable considering the complex sequence of events leading to fertilization. Numerous covariates exist that are essential to successful IVF. It would therefore be ill-advised to consider the normal sperm morphology percentage of a man in isolation from the other parameters. A number of the studies reviewed found other semen parameters, such as motility (Mahadevan and Trouson, 1984; Alper *et al.*, 1985; Hirsh *et al.*, 1986; Ron-El *et al.*, 1991; Barlow *et al.*, 1991; Robinson *et al.*, 1994), motility characteristics (Jeulin *et al.*, 1986; Comhaire *et al.*, 1988; Chan *et al.*, 1989; Enginsu *et al.*, 1992a; De Geyter *et al.*, 1992; Duncan *et al.*, 1993) and concentration (Yovich and Stanger, 1984; Alper *et al.*, 1985; Liu *et al.*, 1988; Liu and Baker, 1988, 1990; Biljan *et al.*, 1994; Calvo *et al.*, 1994; Robinson *et al.*, 1994) also to be positively associated with fertilization *in vitro* and/or pregnancy. Oehninger *et al.* (1988) clinically substantiated the covariation of normal sperm morphology and the insemination concentration, and found that, by increasing the insemination concentration of severe teratozoospermic patients from 100 000 to 500 000 spermatozoa per oocyte, fertilization could be significantly improved.

From this review it is evident that the normal sperm morphology classification system and evaluation procedures used may not be the overriding factors for accurately predicting outcome, as a high proportion of studies using the strict criteria (90.5%; 19/21) as well as the WHO (1980, 1987, 1992) criteria (76.5%; 13/17) obtained positive association with fertilization and/or pregnancy. The most important factors may rather be the level of commitment to use sperm morphology in male factor diagnosis, good inter- and intra-observer and laboratory quality control and the establishment and use of clinically based normal sperm morphology descriptive guidelines and fertility thresholds. Adherence to these basic principles has helped to establish the Tygerberg strict criteria as a dependable diagnostic tool. While the classification system has been refined to include the poor-prognosis (p-pattern (4% normal sperm morphology) and the good-prognosis (g-pattern, 5–14% normal sperm morphology; Kruger *et al.*, 1988) groups, the physiologically based criteria (Menkveld *et al.*, 1990) and clinically based thresholds (Kruger *et al.*, 1986) have remained constant since 1986. The classification system has now been adopted and used successfully by authors world-wide. The majority of the studies (Oehninger *et al.*, 1988; Enginsu *et al.*, 1992a; Ombelet *et al.*, 1994; Hernandez *et al.*, 1996) have confirmed the predictive value of normal sperm morphology within the established thresholds (≤4% and ≤14%). In comparison, the WHO guidelines, which are another of the major classification systems in use world-wide, have changed dramatically since their inception in 1980, becoming 'stricter' with each revision (1987 and 1992). The result has been a high level of subjectivity and a lack of consensus, especially with regards to their clinical value and corresponding fertility thresholds. In a recent publication by Ombelet *et al.* (1997b), a similar demographic distribution of methodologies was obtained from the analysis of questionnaires sent to different laboratories world-wide. In the article they make a plea for the urgent need to standardize sperm morphology methodology to extract maximum value from this important semen parameter.

In all the studies (n = 18) presenting sufficient data for OR evaluation, positive OR (1.27–25.08) were obtained for IVF, with only 16.7% (3/18) not reaching significance. When the data were analysed according to the particular classification system and threshold used, the following did not reach significance: Figueiredo *et al.* (1996) using a 5% (strict criteria) threshold, Yue *et al.* (1995) using a 14% (strict criteria) threshold and Yovich and Stanger (1984) using a 60% (WHO, 1980) threshold. The reasons for the good association between normal sperm morphology and IVF have been shown by studies demonstrating the selective properties of the zona pellucida (Franken *et al.*, 1989; Menkveld *et al.*, 1991) and the oocyte oolemma (Liu and Baker, 1994b). The selection process performed by these physiological agents helped in the initial formulation of the strict criteria, the aim of which is to identify those

spermatozoa with the greatest probability of fertilizing an oocyte.

A lower percentage of the analysed studies produced significant predictive value outcomes when predicting pregnancy than when predicting IVF outcome. This outcome was in no way influenced by the normal sperm morphology threshold used. The reasons for lower number of significant OR outcomes in the prediction of pregnancy may be two-fold: additional variables may have decreased the importance of sperm morphology and/or it may be due to statistical formulation, i.e. relatively small sample sizes and small percentage differences. An important additional variable controlled for by the clinician and having a major influence on pregnancy outcome is the number of embryos transferred. Ten of the 18 studies analysed provided their protocol or the mean number of embryos transferred. All the protocol values and the means given in the studies were equal to or below four embryos transferred. Whereas the later premise may be correct for individual studies, the differences between the combined pregnancy rates for this study should reach significance. The mean combined pregnancy rate for patients with ≤4% normal forms is 15.2% (60/395) compared to 26.0% (355/1368) for patients with >4% normal forms, as calculated from all studies providing pregnancy data (Table III). Another important factor influencing the pregnancy rate per cycle obtained is the number of cycles that produce no embryo transfers. In the 5% (strict criteria) threshold analysis, the no-transfer rate was 24.0% (86/359) in the ≤4% group compared to 7.4% (80/1088) in the >4% group. A similar outcome was obtained when the 14% threshold was used: a 26.4% (73/276) rate was obtained in the ≤14% group, while a 7.3% (35/480) rate was obtained in the >14% group. Patients suffering from severe teratozoospermia may therefore have a one in three chance of not having a transfer.

The question is, how can the percentage of normal forms in a semen ejaculate project its influence to the stage of conception, as we have shown that higher percentages of normal forms can be equated with higher pregnancy rates. The importance of the spermatozoon's contribution to embryo genesis, haploid genome, the centrosome, and the signal to initiate oocyte activation, cannot, however, be underestimated. Three of the studies reviewed (Yovich and Stanger, 1984; Ron-El *et al*., 1991; Parinaud *et al*., 1993) concluded from their analyses that the presence of increased levels of sperm head abnormalities resulted in delayed fertilization and poor embryo quality. In a review, Grow and Oehninger (1995) also speculated that higher incidences of head abnormalities lead to embryos with a lower pregnancy potential. Although the fertilization rate can be enhanced by increasing the insemination concentration, a lower pregnancy rate is obtained for severe teratozoospermic patients. In a retrospective cohort study, Oehninger *et al*. (1996) compared intracytoplasmic sperm injection (ICSI) with high insemination concentration (HIC) in the severe teratozoospermic (<5%) group and found that HIC produced a higher fertilization rate, but that the percentage of high quality embryos was lower, and the implantation rates were lower. Oehninger *et al*. (1996) speculated that this may be attributed to a 'toxic effect' as a result of the presence of high concentrations of spermatozoa and seminal debris, and/or the presence of large numbers of immotile spermatozoa may influence embryo quality and consequently implantation. Dumoulin *et al*. (1992) also showed that embryonic growth was retarded when greater numbers of spermatozoa were used for insemination.

The advancement of infertility treatment with the introduction of the ICSI procedure has made the correct classification of male fertility paramount, to ensure the best cost–benefit ratio. This is especially true in cases of severe male infertility. The ICSI procedure has been shown to produce consistently fertilization rates of between 50 and 70% in severe male factor cases. The mean IVF rates for patients with a normal morphology percentage <5% ranged from 7.9 to 80.8%. This underlines the importance of being able to identify these severe cases so that they can be given the option of ICSI or at least a diagnostic cycle, i.e. a cycle in which half the oocytes are fertilized by ICSI and the other half inseminated.

In conclusion, standard semen analysis with an accurately evaluated normal sperm morphology count still remains an important screening procedure for male fertility. Although normal sperm morphology may be the most significant indicator of male fertility, the other parameters are essential for an accurate diagnosis. The subjective nature of normal sperm morphology evaluation and its consequential variability, even with the 'stricter' approach, requires certain measures to be implemented world-wide. Consensus has to be obtained on what constitutes a functionally normal spermatozoon and which preparation methods are essential for the accurate evaluation of sperm morphology. Laboratories that commit themselves to the evaluation of sperm morphology must ensure that they adhere to these basic principles and implement the necessary training programme and quality control procedures. Computer-aided sperm analysis systems may be able to play an active role in this process of standardization, as a tool to complement the manual evaluation of sperm morphology and as a training tool. The importance of this role will, however, be determined by the development of computer technology and clinical trials to assess the accuracy of computer-generated

normal sperm morphology assessments. The clinical application of normal sperm morphology requires the performance of a study on a reference population to determine the normal sperm morphology threshold points distinguishing fertile and infertile groups. Ombelet *et al*. (1997a) performed just such a study by prospectively comparing a fertile and a subfertile population to define normal values for different semen parameters. Sperm morphology was found to be the most significant indicator for subfertility, with a cut-off value of 10% according to receiver operating characteristic (ROC) analysis and 5% using the 10th percentile of the fertile population. This reaffirmed the possibility of two subfertile populations and the 5% threshold as the lowest point of fertility.

Although a true meta-analysis was not performed, the OR analyses clearly showed the advantage in accurately evaluating sperm morphology. Normal sperm morphology may not be absolute in its prediction of fertilization and pregnancy, but remains the most cost-effective means of diagnosing male fertility and assisting in the formulation of a treatment regimen. The selection of the correct treatment regimen will help to maximize fertilization, transfer and, ultimately, probability of pregnancy.

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