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WHO 5th Edition Strict Criteria vs. Kruger for Normal Morphology Assessment

In the first appendix articles included with this summary (**"Sperm morphologic features as a prognostic factor in in vitro Fertilization** and **Predictive value of abnormal sperm morphology in in vitro fertilization"),** the following description of morphologically normal spermatozoa is provided:

NOTE:
Similarities are marked yellow and differences marked blue.

"In this laboratory, a spermatozoa is considered normal when the head has a smooth, oval configuration with a well-defined acrosome comprising about 40% to 70% of the sperm head. Also, there must be no neck, midpiece, or tail defects and no cytoplasmic droplets of more than one-half the size of the sperm head. In contrast with other authors, we consider borderline forms abnormal".

Kruger TF, Menkveld R, Stander FSH, Lombard CJ, Van der Merwe JP, van Zyl JA, Smith K. Sperm morphologic features as a prognostic factor in in vitro fertilization. Fertil Steril 1986;46:118.

"In patients with acceptable sperm count and motility, two patterns of abnormal morphology, judged with strict criteria, were identified and described. Spermatozoa were considered normal when the head had a smooth oval configuration with a well-defined acrosome involving about 40% to 70% of the sperm head, as well as an absence of neck, midpiece, or tail defects. No cytoplasmic droplets of more than half the size of the sperm head should be present. In contrast to other methods, borderline forms were counted as abnormal. By evaluating sperm morphology with the proposed strict criteria, its predictive value in in vitro fertilization is enhanced".

Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril. 1988 Jan;49(1):112-7.

In the 2010 WHO 5th edition manual, a very similar description of morphologically normal spermatozoa based on strict Kruger criteria can be found:

2.13.1 The concept of normal spermatozoa
By the strict application of certain criteria of sperm morphology, relationships between the percentage of normal forms and various fertility endpoints (time-to-pregnancy (TTP), pregnancy rates in-vivo and in-vitro) have been established (Eggert-Kruse et al., 1996; Jouannet et al., 1988; Toner et al., 1995; Coetzee et al., 1998; Menkveld et al., 2001; Van Waart et al., 2001; Garrett et al., 2003; Liu et al., 2003), which may be useful for the prognosis of fertility.
WHO 5th manual, p. 57.

2.15.1 Classification of normal sperm morphology
The method recommended here is a simple normal/abnormal classification, with optional tallying of the location of abnormalities in abnormal spermatozoa. The criteria overpage should be applied when assessing the morphological normality of the spermatozoon (Kruger et al., 1986; Menkveld et al., 1990; Coetzee et al., 1998).
For a spermatozoon to be considered normal, both its head and tail must be normal. All borderline forms should be considered abnormal.

The head should be smooth, regularly contoured and generally oval in shape. There should be a well-defined acrosomal region comprising 40–70% of the head area (Menkveld et al., 2001). The acrosomal region should contain no large vacuoles, and not more than two small vacuoles, which should not occupy more than 20% of the sperm head. The post-acrosomal region should not contain any vacuoles. Residual cytoplasm is considered an anomaly only when in excess, i.e. when it exceeds one third of the sperm head size (Mortimer & Menkveld, 2001).
WHO 5th manual, p. 67-68.

CONCLUSIONS:

- There is a very high similarity between the original Kruger strict sperm morphology criteria definitions and WHO 5th edition manual guidelines. The latter were updated with some details, however the major requirements remained the same as proposed by Kruger et al (included articles below).
- In the practical semen analysis associated with objective difficulties and subjectivity as emphasized by the WHO 5th ed. manual, the minor differences in definitions of the original Kruger and WHO 5th strict morphology criteria should not have any significant impact on morphology results.

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Predictive value of abnormal sperm morphology in in vitro fertilization

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In patients with acceptable sperm count and motility, two patterns of abnormal morphology, judged with strict criteria, were identified and described. Patients with <4% normal forms and <30% morphology index (summation of normal and slightly amorphous forms) had a fertilization rate of 7.6% of the oocytes (P pattern, poor prognosis). Patients with normal morphology between 4 and 14% had a significantly better fertilization rate of 63.9% of the oocytes ($P < 0.0001$). Cases with >14% normal forms fertilized within the normal range for the laboratory. By evaluating sperm morphology with the proposed strict criteria, its predictive value in in vitro fertilization is enhanced. Fertil Steril 49:112, 1988

Although there is still extensive debate about the role of sperm morphology in in vitro fertilization (IVF),[1-3] the human model has greatly improved the understanding of the significance of this parameter for fertilization and pregnancy outcome.[4,5] In previous publications[1,4] it was noted that if evaluation of normal sperm morphology is done using strict criteria, this parameter has an excellent predictive value of fertilization. In patients with a sperm concentration > 20×10^6/ml and a motility of >30% with a normal sperm morphology of <14%, the fertilization rate was markedly impaired (37% to 47% per oocyte), as opposed to a high fertilization rate (85% to 88%) when normal morphology was >14%.[1,4]

Although there was severe impairment in the fertilization rate, some of these patients still fertilized the human egg; in these cases, a pregnancy was possible.[4]

The purpose of this study was to evaluate patients with normal sperm morphology < 14% to try to establish a morphologic pattern which can differentiate the subgroup that fertilized from the subgroup that did not.

MATERIALS AND METHODS

Forty-five couples were allocated to the study group in a prospective way. All female partners in these couples had tubal infertility, and the males had either been considered normal or had some abnormal parameters by other laboratories evaluations. Twenty-eight patients were stimulated with a combination of hFSH/hMG/hCG (human follicle-stimulating hormone/human menopausal gonadotropin/human chorionic gonadotropin; 62.2%), 13 with hFSH/hCG (28.8%), and 4 with hMG/hCG (8.8%) following protocols previously published.[6] In the Norfolk experience, all of these protocols have demonstrated provision of preovulatory oocytes with identical fertilization rates. All male patients had to have a sperm concentration > 20

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$\times 10^6$/ml and a progressive motility of >30%[4,7] in the basic semen analysis to try to minimize the impact of these two variables on the fertilization rate. The basic semen evaluation was performed after liquefaction of the specimen delivered for IVF insemination using computer analysis (Cellsoft Semen Analysis System, Labsoft Division of Cryo Resources Ltd., NY). Sperm concentration and percentage of normal motility were assessed in this fashion. Two morphology slides were prepared for each patient from the specimen delivered on the day of laparoscopy for in vitro insemination after liquefaction and were stained by the quick-stain technique.[8] Special care was taken to clean the slides thoroughly with 70% ethyl alcohol before using them, and no more than 5 μl of semen were used in order to make the smears as thin as possible. The slides were air-dried at room temperature, were fixed for 15 seconds with Diff-Quik fixative (Diff-Quik AHS del Caribe, Inc. Aguada, PR 00602) (1.8 mg/l triarylmethane methyl alcohol) prior to staining with Diff-Quik solution 1 (1 gm/l xanthene in sodium azide-preserved buffer) for 10 seconds, and then with solution 2 (0.625 gm/l azure A and 0.625 gm/l methylene blue in buffer) for 5 seconds. In between the fixing step and each of the staining steps, the excess solutions were drained from the slides by blotting the slide edges on bibulous paper. The slides were read on the same day and documented. The morphology was evaluated, as outlined in detail by Kruger et al,[1] by two independent observers, each unaware of the results obtained by the other. This method of evaluation has an inter-technician coefficient of variation and an intra-technician variability that are not significant (Spearman's rank correlation coefficient, $r = 0.8695$ and 0.9650, respectively).[8,9]

Spermatozoa were considered normal when the head had a smooth oval configuration with a well-defined acrosome involving about 40% to 70% of the sperm head, as well as an absence of neck, midpiece, or tail defects. No cytoplasmic droplets of more than half the size of the sperm head should be present.[1] The length of a normal sperm head was 5 to 6 μm and the diameter 2.5 to 3.5 μm (Fig. 1). A micrometer in the eyepiece of the microscope was used to do the routine measurements. In contrast to other methods,[10] borderline forms were counted as abnormal. At least 200 cells per slide were evaluated. The amorphous-head group was divided into two categories: slightly amorphous and severely amorphous. Slightly amorphous forms were those sperm with a head diameter of 2.0 to 2.5 μm, with slight abnormalities in the shape of the head, but with a normal acrosome (Fig. 1). Severe amorphous head abnormalities were defined as those with no acrosome at all or those with an acrosome smaller than 30% or larger than 70% of the sperm head (Fig. 1). Completely abnormal shapes also were put into this category (Fig. 1). Neck defects were also classified into two categories: slightly amorphous and severely amorphous neck defects. The slight neck defects referred to those sperm with debris around the neck or a thickened neck, but with a normally shaped head (Fig. 1). The severe defects referred to those sperm with a bend in the neck or midpiece of more than 30% or a severely amorphous head shape, as described. All other abnormal sperm forms—round, small, large, tapered, double head, double or coiled tail, cytoplasmic droplets—were classified following the World Health Organization classification.[11]

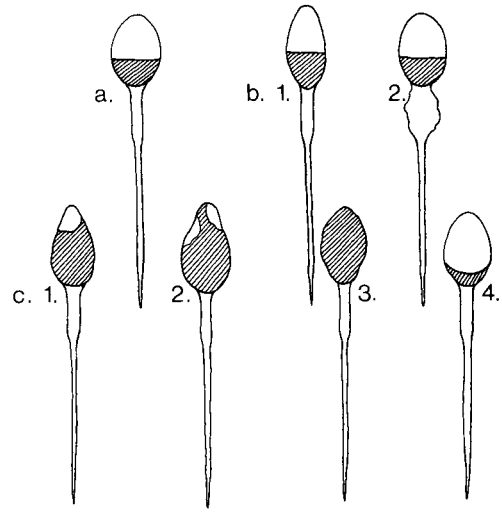

Figure 1 Diagramatic representation of quick-stained spermatozoa. a. Normal form; head, oval shape, smooth configuration, acrosome 40% to 70%, no neck, midpiece, or tail defects. Head length; 5 to 6 μm, diameter 2.5 to 3.5 μm. b. 1. Slightly amorphous head; slightly elongated, loss of oval shape, acrosome 40% to 70%, diameter 2 to 2.5 μm. b. 2. Slightly amorphous neck defect; thick neck but normal-shaped head. c. Severe amorphous forms; abnormalities in shape and acrosome. c. 1, 2. Abnormally small acrosome. c. 3. No acrosome. c. 4. Acrosome > 70% of head.

Female or male patients with antisperm antibodies were excluded from this study.

The human IVF procedures used for sperm preparation, insemination, and culture in the Norfolk program have been described previously.[12] Only mature oocytes with an extruded polar body were used in this study; 50,000 to 100,000 sperm/ml/egg were used for oocyte insemination in a total of 3.0 ml of insemination medium.

After completion of the study, the results of the hamster tests in patients that had the assay performed at least 8 weeks before the IVF procedure were evaluated. The hamster test was performed as outlined by Swanson et al.[13] Penetration > 20% was considered good, between 11 and 19% doubtful, and <10% poor. The donors used as control always penetrated above the 20% level.

The percentage of normal morphology, the concentration, and motility were noted carefully in each case, as were the fertilization and cleavage rates and pregnancy outcome. The relationships between the sperm parameters and the fertilization rates were examined using multiple regression analysis in the Statistical Analysis System (SAS) general linear model (GLM) procedure. The SAS GLM procedure allows examination of all submodels of the complete multiple regression model. The multiple regression analysis examines the contribution of all the independent variables to the variation in the dependent variable fertilization. Fertilization was standardized for the number of preovulatory eggs by dividing the number of eggs exhibiting fertilization by the number of preovulatory eggs. Pregnancy rate per laparoscopy was calculated by dividing the total number of laparoscopies by the number of pregnancies in the study. Pregnancy rate per embryo transfer was calculated by dividing the number of patients who reached the transfer stage by the number of pregnancies. The following variables were evaluated: percent normal sperm morphology, amorphous head abnormalities (slight and severe), neck abnormalities (slight and severe), small, large, round, tapered, and double heads, cytoplasmic droplets, and tail abnormalities (double and coiled).

RESULTS

Of the 45 patients included in this study, 13 (28.9%) did not fertilize any oocytes at all, 17 (37.8%) fertilized <50% of the oocytes obtained, and 15 (33.3%) fertilized >50% of oocytes obtained. This should be compared with a fertilization rate for patients with tubal infertility of 89% to 92% in our laboratory.

The patients were divided into two groups: group I, 14 patients (no fertilization) and group II, 32 patients (fertilization of at least one oocyte). The mean sperm concentration in group I was 63.3 ± 42.8 × 10^6/ml (mean ± standard deviation) and in group II, 83.3 ± 57.8 × 10^6/ml (no significant difference) (Table 1). The mean motility in group I was 45.6 ± 13.2% and in group II, 55.3 ± 18.6% (no significant difference) (Table 1). By eliminating abnormal concentration and abnormal motility, we tried to individualize and define the effect of morphology and its abnormalities in the process of fertilization. There was a significant difference between the percent of normal morphology (1.8 ± 2.4% in group I and 7.7 ± 3.3% in group II; $P < .0001$) and the percentage of slightly amorphous abnormalities (head and neck), which was 18.0 ± 10.9% in group I and 34.3 ± 6.7% in group II ($P < .0001$; Table 2). None of the other variables showed a significant difference between the two groups.

Table 1 Abnormal Morphology as a Predictor of Human IVF: Semen Analysis

	Group 1 (n = 13)	Group II (n = 32)
Normal forms (%)	1.8 (2.4)	7.7 (3.3)[a]
Slightly amorphous (head and neck)	18.0 (10.9)	34.3 (6.7)[a]
Morphology index (slightly amorphous forms and normal)	19.7 (11.7)	42.0 (7.8)[a]
Concentration (million/ml)	63.3 (42.8)	83.3 (57.8)[b]
Motility (%) Mean (standard deviation)	45.6 (13.2)	55.3 (18.6)[b]

[a] $P < 0.0001$.
[b] Not significant.

The predictive value of normal morphology ($r^2 = 0.44$) was better than that of slightly amorphous forms ($r^2 = 0.36$). When we added the percent of normal morphology and slightly amorphous abnormalities (morphology index) and performed regression analysis, there was a highly significant correlation between that index and fertilization ($P < .0001$; Table 1), with an even better predictive value ($r^2 = 0.56$). The mean for morphology index was 19.7 ± 11.7% in group I and 42 ± 7.8% in group II (Table 1).

The SAS general linear model was used with the number of embryos as the dependent variable to determine a threshold to indicate where the chances of fertilization were significantly impaired. A threshold of 4% was indicated for normal morphology and 30% for the combination of normal morphology and slightly amorphous forms (morphology index). The fertilization rate per oocyte in group I (morphology index <30%, normal morphology <4%) was 7.6% and in group II (morphology index >30%, normal morphology >4%) was 63.9% (Table 2).

The mean number of embryos in the 13 patients in group I was 0.4 and for the 32 patients in group II

Table 2 Abnormal Morphology as a Predictor of Human IVF: Fertilization Rate

45 Patients, conc. > 20 million, motility > 30%

	Group I (n = 13)	Group II (n = 32)
	P pattern	*G pattern*
Fertilization rate (%) (per oocyte)	7.6	63.9[a]
Mean no. embryos (per patient)	0.4	2.6[a]

[a] $P = 0.0001$.

was 2.6; these means were significantly different ($P < 0.0001$; Table 2). Patients were followed with β-hCG, estradiol, and progesterone determinations on a weekly basis, pelvic ultrasound starting on the seventh week after the last menstrual period, and clinical evaluation to determine pregnancy status and type of gestation. The pregnancy rate in group I was 1 out of 13 patients (7.6%) and in group II was 10 out of 32 patients (31.2%). The ongoing pregnancy rate in group I was 1 out of 13 patients (7.6%) and in group II, 6 out of 32 patients (18.7%), with three clinical miscarriages and one ectopic pregnancy. The differences between these two groups in terms of reproductive performance did not reach statistical significance because of the small number of patients.

Of the 45 patients studied, 14 had a hamster test performed prior to the IVF procedure. All 14 had a penetration rate below 10%. Four out of the 14 (28.6%) patients with poor penetration rates did not fertilize any oocytes in vitro, but 10 of 14 patients (71.4%) did fertilize in vitro. Five of 10 patients (50%) fertilized >50% of the eggs, and 5 (50%) fertilized <50% of the eggs.

DISCUSSION

Normal morphology evaluated by strict criteria is a valuable tool to predict a patient's chance to fertilize and to reach the transfer stage. In a previous study performed at the Jones Institute,[4] 70 of 71 patients with normal morphology > 14% reached the transfer stage, reflecting a high fertilization rate in this group. If the normal morphology is <14%, the fertilization rate per oocyte is markedly impaired.[1,4] This study was designed to evaluate the sperm morphology in this group and to try to define morphologic patterns in patients with and without fertilization. Our results indicate that severe impairment of fertilization will take place at a level of <4% normal morphology, based on the strict criteria explained previously (Table 2). Results also indicate that by adding the slightly amorphous forms to the normal forms, a "morphology index" can be established with a cutoff figure at the 30% level. Patients with a value of <30% morphology index will have a severe reduction in fertilization as compared with patients having an index > 30% ($P < 0.0001$) (Table 2). None of the other semen parameters evaluated were of any help to predict a patient's chance to fertilize.

The advantage of strict morphology evaluation is the fact that it is reproducible between patients and between different technicians performing the test.[1,8] It also allows the clinician to classify the patient into one of two specific groups (<14% and >14% normal morphology), giving a reliable criterion that can be used to counsel the patient and to plan the approach in future IVF cycles.

Based on the significant differences between normal morphology and the slightly amorphous forms in groups I and II, we propose that two patterns can be observed in the <14% normal morphology group. The P pattern (poor prognosis pattern) has a mean normal morphology of 1.8% and mean slightly amorphous forms of 18%, with a morphology index < 30% (Table 1). The G pattern (good prognosis pattern) gives the patient a significantly better chance to fertilize ($P < 0.0001$) than the P pattern (Table 2). The mean normal morphology in the G pattern was 7.7%, the mean slightly amorphous forms were 34.3%, with a morphology index > 30%. Based on these patterns, predictions on chances of fertilization can be done with much more accuracy in the group with <14% normal morphology.

The fertilization rate for all patients with a normal morphology < 4% and morphology index < 30% (P pattern) was 7.6%; when the normal morphology was >4% and the morphology index > 30% (G pattern), the fertilization rate was 63.9%. Only one pregnancy was established in patients with a P pattern; in patients with a G pattern, the ongoing pregnancy rate was 60%, which compares favorably with the ongoing pregnancy rate previously reported in our overall population.[14] This observation again confirms previous reports[15,16] that if fertilization occurs, the performance of the embryos, as well as the transfer and pregnancy rates, are no different from the general IVF population.

The question now arises whether the fertilization rate and prognosis of patients with normal morphology < 14% can be improved, especially in those with a P pattern, but also in those with a G pattern who fertilized <50% of the oocytes. Can they per-

haps benefit by simply increasing the concentration of sperm per milliliter of the insemination medium at the time of IVF from 50,000/ml to 500,000/ml? There have been several reports warning against a significant decrease in fertilization rates in vitro in mice and hamsters when the sperm concentration was increased.[17,18] This decrease can be due to excessive numbers of antifertilization factors[19] or proteases[20] near the oocyte. Nevertheless, in the beginning of our own program, insemination was done routinely with 500,000 sperm/ml/egg and the fertilization rate was no different. It also was demonstrated in studies with mice, using suboptimal concentrations of sperm, that as the sperm density is reduced, fertilization rates also are reduced.[19] To answer these questions, a prospective study is being conducted.

Another important point in male factor cases is the timing of insemination. In Norfolk the extrusion of the polar body is used as an indicator of oocyte maturity,[12] at which time insemination takes place.

The correlation of the sperm penetration assay (SPA) and IVF was not good in this study, with 28.6% no fertilization and 71.4% fertilization rate per patient with <10% SPA penetration in all 14 cases. In a previous study[21] this group indicated that there is a good correlation between normal morphology and SPA penetration rate. If normal morphology was <14%, 85% of cases did not penetrate above the 10% level. If normal morphology was >14%, the penetration rate above the 10% level was 86% ($P < 0.0001$). We conclude from these observations that the SPA is giving us the same information as normal morphology greater or lesser than 14%[1,4] in the population studied. A SPA < 10% and a normal morphology < 14% are parameters warning the clinician of potential problems in IVF due to the male factor. Without identifying the different patterns (P and G), valuable predictive information will be lost.

It is worth emphasizing that these criteria are useful only in IVF with the techniques used in Norfolk. The significance of these abnormalities in clinical practice remains to be demonstrated.

The evaluation of sperm morphology is a controversial issue. Results in fertilization rates in IVF units differ.[1,2,4,16] Do we look at the same spectrum of abnormalities, explaining the difference in results, or is our classification of abnormally and normally shaped sperm in need of revision? It is our opinion that the latter is true and thus needs the attention of those involved in the field.

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Sperm morphologic features as a prognostic factor in in vitro fertilization

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To determine whether there is a prognostic value in the percentage normal sperm morphologic features in a human in vitro fertilization (IVF) program, the authors conducted a prospective study in women with bilateral tubal damage. Based on the percentage of morphologically normal spermatozoa, the patients were divided into four groups: group I, normal morphologic features between 0% and 14%; group II, 15% to 30%; group III, 31% to 45%; and group IV, 46% to 60%. One hundred ninety successful laparoscopic cycles were evaluated. In group I, 104 oocytes were obtained, of which 37% fertilized, but no pregnancy resulted; in group II, 81% of 324 oocytes were fertilized, with a pregnancy rate per embryo transfer (ET) of 22%; in group III, 82% of 309 oocytes were fertilized, with a 31% pregnancy rate; and in group IV, 91% of 69 oocytes were fertilized, with a pregnancy rate of 12%. Probability models indicated that there was a clear threshold in normal sperm morphologic features at 14%, with high fertilization and pregnancy rate in the groups with normal sperm morphologic features > 14%. Fertil Steril 46:1118, 1986

Sperm count, motility, and the percentage normal morphologic features have been the traditional criteria for semen quality. In 1976 Van Zyl et al.[1] proposed a reclassification of the criteria for "normal" semen parameters. The in vitro penetration of zona-free hamster eggs by human spermatozoa has become a valuable new tool in the assessment of human semen,[2] but this test is not easy to perform, and the results have not always been consistent between laboratories. Rogers et al.[3] analyzed sources of variability in the assay and stressed quality control.

Evaluation of the percentage normal sperm morphologic features is subjective and difficult to compare between different laboratories throughout the world. Different means of assessing normal sperm morphologic features have been described.[4, 5] Although it is difficult to compare the morphologic features, the critical issue is what the morphologic features actually tells us in a specific laboratory or clinic. To answer the question of whether there is a prognostic value in this parameter regarding the fertilization and preg-

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nancy rate in a human in vitro fertilization (IVF) program, we conducted a prospective study.

MATERIALS AND METHODS

PATIENTS

The women accepted into the program had bilateral tubal damage, diagnosed with the use of laparoscopy and hysterosalpingography. Their male partners had four semen analyses before the women were accepted into the IVF program.

The semen analysis methods used in our laboratory were described in detail by Van Zyl.[6, 7] The methods used for determination of the three variables used in this study are, in short, as follows. The semen samples were obtained after 3 to 4 days of abstinence by masturbation at the laboratory. Immediately after liquefaction, a drop of the well-mixed specimen was placed on a clean and prewarmed glass slide at 37°C, covered with a cover slip, and left for a few minutes. The microscope was provided with a hot stage to keep the slides at 37°C. The preparation was examined under a magnification of both × 10 and × 40 objectives. The quantitative motility or percentage of motile spermatozoa and qualitative motility or speed of forward progression was assessed in at least ten separate randomly selected high-power fields, as described by MacLeod.[8] At the same time, presence of agglutination and particulate debris was observed and an estimation of the sperm concentration made. The viability (i.e., percentage of live and dead spermatozoa) was determined with the use of supravital staining.[9, 10] Depending on the estimated sperm concentration, a 1/10, 1/20, or 1/100 dilution of the semen sample was made with the use of a glass tuberculin syringe, instead of a white blood cell pipette.[11] An improved, double-ruled Neubauer hemocytometer (Assistent, FRG) was used for counting the spermatozoa. Two dilutions were made for every sample. The difference between the two dilutions for each sample was not > 10% for low concentrations and not > 20% for concentrations of > 60 × 10^6/ml.[12]

The following procedures were used for the assessment of the morphologic characteristics of the spermatozoa. The slides were thoroughly cleaned, washed in alcohol, and dried before use. For a good, reliable, and repeatable assessment, a thin and well-spread smear was made so that each spermatozoa could be clearly and individually visualized. The smears were air-dried and on the following day fixed and stained according to the Papanicolaou[13] method. The morphologic classification used in the Tygerberg hospital unit is based on a modification of the methods described by MacLeod[14] and Eliasson.[12] This system takes the whole spermatozoa, as well as the presence of germinal epithelial cells, into consideration.

In this laboratory, a spermatozoa is considered normal when the head has a smooth, oval configuration with a well-defined acrosome comprising about 40% to 70% of the spermhead. Also, there must be no neck, midpiece, or tail defects and no cytoplasmic droplets of more than one-half the size of the spermhead. In contrast with other authors,[12, 15] we consider borderline forms abnormal. At least 100, but preferably 200, spermatozoa with tails were classified into one of seven groups: normal (head and tail normal), normal head but with an other abnormality present, large heads, small heads, tapering heads, duplicated heads or amorphous heads all with or without tail, neck or midpiece defects. Tail, neck, and midpiece defects, loose head, immature germinal cells, and unknown cells were recorded separately and reported per 100 spermatozoa. The size of the spermatozoa were evaluated in five different areas to ensure a more randomized evaluation.

All of the men had a normal spermatozoa concentration of ≥ 20 × 10^6/ml, normal motility of ≥ 30%, and a normal forward progression of ≥ 2.0. In some of the patients, the percentage normal sperm morphologic features was < 20%. On the basis of previous experience, we prospectively divided all of the men into four groups based on the percentage normal morphologic features evaluated on the day of insemination in the IVF cycle. In group I the percentage normal morphologic features was 0% to 14%; in group II, 15% to 30%; in group III, 31% to 45%, and in group IV, 45% to 60%.

The semen samples were obtained 2.5 hours before insemination and prepared as follows: 1 ml semen was diluted with 2 ml of Ham's F-10 medium (GIBCO, Grand Island, NY) and washed twice with centrifugation at 200 × *g* for 10 minutes. After the final wash, the supernatant was discarded and 1 ml of medium was layered over the pellet. The tube was placed in the incubator at 37°C for 30 minutes. A count was performed after 30 minutes and the motility recorded.

All of the women received a combination of clomiphene citrate (CC) and human menopausal

Table 1. *Number of Cycles, Cycles with No Fertilized Oocytes, Cycles with Embryo Transfer, Cycles with Pregnancies, Pregnancy Rate per Successful Laparoscopy, and Pregnancy Rate per Embryo Transfer*

	Group I (0%–14%)	Group II (15%–30%)	Group III (31%–45%)	Group IV (46%–60%)	Total
Cycles observed					
No.	22	83	67	18	190
%	12	44	35	9	
Cycles with 0 fertilized oocytes					
No.	11	6	3	1	21
%[a]	50	7	4	6	
Cycles with embryo transfer					
No.	10	72	62	17	161
%[b]	45	87	93	94	
Cycles with pregnancies	0	16	19	2	37
Pregnancy rate per successful laparoscopy (%)	0	19	28	11	19
Pregnancy rate per embryo transfer (%)	0	22	31	12	23

[a]Percentage of cycles in group with no fertilized oocytes.
[b]Percentage of cycles in group with embryo transfer.

gonadotropin (hMG) as outlined previously.[16] The oocyte recovery took place 36 hours after 10,000 U of human chorionic gonadotropin (hCG) was injected. Each oocyte was incubated in 1.5 ml of Ham's F-10 medium with 10% patient's serum in a Petri dish (Falcon Plastics 3037, Oxnard, CA) for 5 to 6 hours. Insemination took place with 100,000 spermatozoa/ml of insemination medium. Fertilization was recorded after 12 to 16 hours if two pronuclei could be detected and, finally, if cleavage occurred. A pregnancy was defined as a β-hCG, which doubled from day 10 to 12 and had to be confirmed at 7 to 8 weeks with the use of ultrasound examination. The pregnancy rate was computed by dividing the number of pregnancies by the number of successful laparoscopies and embryo transfers (ET).

RESULTS

Two hundred five laparoscopies were performed, and 190 successful laparoscopic cycles in 129 patients were evaluated. (In these cycles, oocytes were obtained.) Eighty-six patients had only one cycle, 30 had two cycles, 9 had three cycles, 3 had four cycles, and 1 had five cycles repeated.

In group I (morphologic features 0% to 14%) 22 cycles, in group II 83 cycles, in group III 67 cycles, and in group IV 18 cycles were observed (Table 1). In group I, 104 oocytes were obtained; of these, 37% fertilized. In group II there were 324 oocytes, with a fertilization rate of 81%; in group III, 309 oocytes, with a fertilization rate of 82%; and in group IV, 69 oocytes, with a fertilization rate of 91% (Table 2).

In group I, 45% of patients with a successful laparoscopy reached the ET stage; in group II 87%; in group III 93%; and in group IV 94% (Table 1).

The pregnancy rate per ET was 0% in group I; 22% in group II; 31% in group III; and 12% in group IV (Table 1). In five couples with repeated cycles, the man was noted to have values both below and above the threshold of 14% normal sperm morphologic features (Table 3). The mean sperm concentrations and motility are shown in Table 4.

Logistic regression was used to investigate the associations of certain variables with pregnancy outcome.

Table 2. *Fertilization Rate per Oocyte*

	Group I (0%–14%)	Group II (15%–30%)	Group III (31%–45%)	Group IV (46%–60%)	Total
Total oocytes					
No.	104	324	309	69	806
%	13	40	38	9	100
Oocytes fertilized	38	264	252	63	617
Fertilization rate/oocyte (%)	37	81	82	91	77

Table 3. *Patient Couples with Normal Sperm Morphologic Features Measurements Below and Above the Threshold of 14% Having Repeated Laparoscopies*

Patient	Cycle	% Normal sperm morphologic features	No. of oocytes	No. fertilized	No. transferred	Pregnant[a]
23	1	5	1	0	0	0
	2	38	5	4	3	0
	3	20	4	4	4	1
40	1	16	2	1	1	0
	2	9	3	3	2	0
	3	16	4	4	4	1
51	1	30	3	3	3	0
	2	12	9	0	0	0
	3	35	5	5	3	1
55	1	12	6	0	0	0
	2	50	6	6	5	0
	3	40	8	8	5	0
64	1	20	5	5	5	0
	2	13	4	2	2	0

[a]Pregnancy = 1, no pregnancy = 0.

Spearman correlations between the first and second cycle for morphologic features and number of oocytes for the 43 patients who had first and second laparoscopies were $r = 0.223$, $P = 0.15$ and $r = 0.052$, $P = 0.74$, respectively.

Because the correlations between the repeated cycles were not significant, we considered the 190 cycles independent observations in the probability analysis.

The percentage normal sperm morphologic features and the number of oocytes representing the female factor were the two variables used to investigate the associations with the probability of pregnancy. The (0-1) outcome of pregnancy was considered in the following way: 0 represented failure and 1 represented success.

MODEL A

The logistic regression showed that the male factor, percent normal sperm morphologic features, had a significant nonlinear association and the female factor, number of oocytes, a significant linear association with the probability of pregnancy (model chi-square = 12.95 with 3 degrees of freedom, $P = 0.0047$).

The nonlinear association of morphologic features is of interest if one considers the plot of outcomes in Figure 1. There is a clear threshold in percent normal sperm morphologic features at 14%. In the interval of 0% to 14% normal sperm morphologic features, the number of oocytes varied between 1 and 13, but no pregnancy was obtained in this group. This threshold, together with the absence of any pregnancies in the 50% to 60% interval in normal sperm morphologic features, is the reason for the significant nonlinear association.

What happens between the male and female factors above the natural threshold? To investigate this we modeled a subset of 168 cycles, all falling above the threshold of 14% normal sperm morphologic features.

MODEL B

Logistic regression with a backward elimination procedure was used. All of the variables of model A were presented to the modeling procedure and those that were no longer significantly associated with the probability of pregnancy were eliminated. The result was that the number of oocytes was the only variable that still had a significant linear and positive association with the probability of pregnancy (model chi-square = 5.34 with a 1 degree of freedom, $P = 0.0208$).

Table 4. *Mean Sperm Concentration Count and Motility*

	Group I (0%–14%)	Group II (15%–30%)	Group III (31%–45%)	Group IV (46%–60%)	Total
	mean ± SD	*mean ± SD*	*mean ± SD*	*mean ± SD*	*mean ± SD*
Count (× 10/ml)	53 ± 22.3	78.2 ± 55	84.3 ± 33.6	86.5 ± 39	78.2 ± 44.7
Motility (% motile)	41.8 ± 11.4	49.0 ± 9.3	50.4 ± 9.9	55 ± 9.8	49.3 ± 10.3

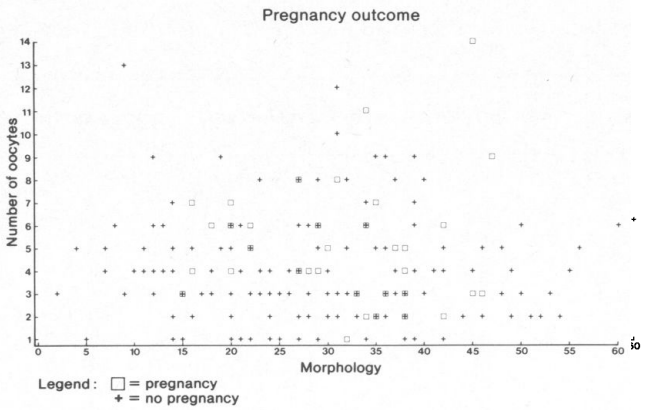

Figure 1
Scatterplot of number of oocytes and % normal sperm morphology by pregnancy outcome.

From the results of models A and B it can be deduced that threshold of morphologic features plays a significant role in the probability of a pregnancy.

The observed proportion of pregnancies below and above the threshold for various numbers of oocytes are given in Table 5.

DISCUSSION

In the Tygerberg Hospital Unit, > 20% normal sperm morphologic features is considered normal, and if all semen parameters are normal such patients are probably fertile.[1] Van Zyl et al.[1] observed this tendency when they analyzed the pregnancy rate in the Infertility Clinic at Tygerberg Hospital. One of the reasons why the results in this unit differ from the World Health Organization's criteria could be the very strict analysis of morphologic features where borderline forms are considered abnormal.

In only five couples with repeated cycles was the percentage normal sperm morphologic features below and above the threshold. The chance that this intraindividual variation in the percentage of the normal sperm morphologic features could be due to a laboratory fault must be regarded as small. Studies have shown that with the strict criteria used in this laboratory our evaluation of morphologic features has a high degree of accuracy and precision (Menkveld, unpublished data). This will be discussed in another publication. In three couples (23, 51, and 55) in whom the percentage was ≤ 14%, no fertilization occurred (Table 3).

There is a clear threshold in the percent normal sperm morphologic features at 14% in this study (Fig. 1). The fertilization rate per oocyte was 37% in group I, in which the percent normal sperm morphologic features was < 14%; in groups II, III, and IV combined, the rate was 84.6% per oocyte. Mahadevan and Trounson[17] also indicated that the percentage of abnormal sperm forms was significantly related to the fertilization rate. The chances of patients with successful laparoscopies reaching the ET stage in group I was also reduced to 45%, whereas in the other groups combined, 91% reached the ET stage.

The zona-free hamster egg test is used to test the ability of the human sperm to penetrate the ooplasm. Rogers et al.[2] indicated that the morphologic factor plays in important role in the fertilization process; 73.7% of infertile men can have a normal count and motility but have lower-than-normal morphologic features. This was also the experience in this unit. If the importance of abnormal morphologic features is not appreciated, these patients can be considered fertile or as cases of unknown infertility. The ability to evaluate morphologic features is subject to experience and a strict protocol in the laboratory, as outlined above. Morphologic features are often judged by laboratory personnel without sufficient experience or background.

The observed proportions of pregnancies given by the results of this study indicate that in the group in whom the percentage normal sperm morphologic features is ≤ 14%, irrespective of the number of oocytes obtained, no pregnancy resulted (the 95% confidence limits are 0% to 16%). In the groups with normal sperm morphologic features > 14%, the female factor (e.g., the number of oocytes obtained) plays an important role in the chances of a pregnancy. If only one to two oocytes are obtained, the chance of a pregnancy

Table 5. *Observed Proportion of Pregnancies Below and Above the Threshold of Percentage Normal Sperm Morphologic Features*

% Normal sperm morphologic features	No. of oocytes	Observed proportion of pregnancies		95% confidence limits
		No.	%	
≤ 14%	1–13	0/22	0	0–0.16
> 14%	1–2	5/44	11.4	0.020–0.20
	≥ 3	32/124	25.8	0.181–0.335

per successful laparoscopy is 11.4% (with 95% confidence limits [2% to 20%]). However, if more than two oocytes are obtained, the pregnancy rate per successful laparoscopy is 25.8% (with 95% confidence limits [18.1% to 33.5%]).

The implication of the lower fertilization rate in the group with ≤ 14% normal sperm morphologic features is of practical relevance for the clinician and the patient. It is important to give the patients in an IVF program a realistic view of their prognosis. There is an excellent correlation with the percentage normal sperm morphologic features and fertilization and pregnancy rates. This correlation was also pointed out by Rogers et al.[2] and Aitken et al.[18] in a group of patients with unexplained infertility. Aitken et al. do not agree that there is a good predictive value, in spite of the statistical association.[18] However, we are convinced that the percentage normal sperm morphologic features has an important role in the fertilization and pregnancy rate in the human in vitro model. The evaluation of normal sperm morphologic features is a routine laboratory procedure at Tygerberg Hospital, and it has a high precision and prognostic value.

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