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The relationship between sperm morphology and rates of fertilization, pregnancy and spontaneous abortion in an in-vitro fertilization/intracytoplasmic sperm injection programme

Kersti Lundin[1], Brita Söderlund and Lars Hamberger

Department of Obstetrics and Gynecology, University of Göteborg, Sahlgrenska Hospital, S-413 45 Göteborg, Sweden

[1]To whom correspondence should be addressed

The morphological normality of a spermatozoon is considered to be an important factor in relation to its ability to fertilize an oocyte. We examined the influence of morphology (strict criteria) on the rates of fertilization, pregnancy and spontaneous abortion obtained following conventional in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) in our clinical programme. We found our fertilization cut-off values for conventional IVF to be slightly different from those of the Kruger group (10 and 5%, compared to 14 and 5%). We also found the pregnancy rate per transfer to be as good or better in the groups with <5% normal forms: 36% of these men had a fertilization rate >50% using conventional IVF, showing that fertilization capacity is not necessarily impaired even in this 'poor prognosis' group. With the exception of the ICSI group with 5–9% normal forms, the rate of spontaneous abortion in this study was similar to or lower than in our IVF/ICSI programme overall. When the 5–9% normal spermatozoa group was divided into those with teratozoospermia as the only factor and those with additional sperm factors, the increased abortion rate was found in the group with multiple sperm factors (67% spontaneous abortions).

Key words: human/in-vitro fertilization/sperm morphology/strict criteria

Introduction

Several different criteria are utilized for determining the morphology of spermatozoa. The most widespread are the World Health Organization criteria (WHO, 1992) and the more recently introduced Tygerberg strict criteria (e.g. Kruger *et al.*, 1987, 1988; Menkveld *et al.*, 1990; Yang *et al.*, 1995), where all borderline forms are considered abnormal. Enginsu *et al.* (1991) found that evaluation according to strict criteria was more effective in predicting fertilization *in vitro* than the WHO criteria, while a study by Morgentaler *et al.* (1995) found the traditional WHO criteria to be more predictive, especially of poor IVF outcome. However, it seems that the best predictive value is obtained when a combination of sperm morphology, percentage progressive motility and total motile sperm count is evaluated (Enginsu *et al.*, 1992; Grow *et al.*, 1994). Several groups have found that poor morphology, poor motility and a low concentration often occur simultaneously (Haidl *et al.*, 1993; Grow *et al.*, 1994). It has also been shown in a number of studies that semen samples with a low number of morphologically normal spermatozoa produce lower fertilization rates when used for conventional in-vitro fertilization (IVF) (Kruger *et al.*, 1988; Oehninger *et al.*, 1988; Enginsu *et al.*, 1991; Grow *et al.*, 1994; Robinson *et al.*, 1994; Vawda *et al.*, 1996). In the study by Grow *et al.* (1994) it was found that progressive motility and total motile sperm count had no influence on the fertilization if the morphology score was >14% normal forms. On the other hand, Robinson *et al.* (1994) showed that the majority of patients, even with <5% normal forms, achieved good fertilization in conventional IVF as long as the sperm concentration and motility were within the normal range according to WHO standards. Most studies have also found a lower pregnancy rate for the poor morphology group (Oehninger *et al.*, 1988; Kobayashi *et al.*, 1991; Grow *et al.*, 1994; Ombelet *et al.*, 1994), although in the study by Yue *et al.* (1995), the group with <5% normal spermatozoa obtained the highest pregnancy and implantation rates.

According to the Tygerberg strict criteria, the sperm samples were originally divided into three morphological categories: >14% (=normal group), 5–14% (=good prognosis group) and <5% (=poor prognosis group) normal forms. In a study performed by Kruger *et al.* (1988), they found that in their system a significantly lowered fertilization rate was seen when <14% normal forms were found in the ejaculate, and this group was considered to be 'subfertile', while <5% normal forms resulted in an even lower fertilization rate.

A few recent studies have shown that morphology does not seem to correlate with the fertilization rate when using intracytoplasmic sperm injection (ICSI) (Küpker *et al.*, 1995; Mansour *et al.*, 1995; Nagy *et al.*, 1995; Svalander *et al.*, 1996). This method, where a single spermatozoon is deposited inside the cytoplasm of the oocyte, makes it possible to fertilize the oocyte using a spermatozoon that would never be able to penetrate the zona pellucida and/or the oolemma by itself, e.g. an immotile spermatozoon, a spermatozoon with no acrosome, or a spermatozoon with a very disturbed morphology. The remaining features required of the sperm cell are to be able to decondense its nucleus inside the ooplasm and to retain its chromosomal DNA intact.

To date, it has not been possible to correlate poor sperm morphology with damaged sperm DNA (Martin and Rademaker, 1988; Rosenbusch *et al.*, 1992). However, Cohen *et al.* (1991b) and Parinaud *et al.* (1993) found that poor sperm morphology resulted in poor embryo quality in their systems, and there is speculation as to whether IVF with sperm samples

Table I. The concentration and motility (mean and range) of the sperm samples utilized in this study, categorized according to the percentage of morphologically normal spermatozoa

	In-vitro fertilization % normal spermatozoa			Intracytoplasmic sperm injection % normal spermatozoa		
	<5% (n = 22)	5–9% (n = 88)	>10% (n = 355)	<5% (n = 26)	5–9% (n = 70)	>10% (n = 61)
Concentration ($\times 10^6$/ml)	23 (20–300)	62 (20–200)	88 (20–350)	25 (2–90)	28 (2.5–100)	65 (1–400)
Motility (%)	43 (30–60)	48 (30–60)	49 (30–80)	39 (10–80)	46 (7–80)	44 (5–70)

of very poor morphology, apart from resulting in a poor fertilization rate, also produces a higher rate of spontaneous abortion (e.g. Oehninger *et al.*, 1988).

In the present study, we evaluated our results from 2 years of using the Tygerberg strict criteria. We attempted to correlate sperm morphology with the rates of fertilization, pregnancy and spontaneous abortion, both for conventional IVF and for ICSI cycles. We also compared the ICSI results from sperm samples with poor morphology as the only factor (pure teratozoospermic group) with those from samples that suffered from one or two additional factors (oligozoospermia and/or asthenozoospermia).

Materials and methods

The study population consisted of 622 patients who participated in our IVF programme during a 2-year period. Altogether, results from 465 conventional IVF cycles and 157 ICSI cycles were evaluated.

From the conventional IVF programme, all patients with a sperm sample of $\geqslant 20\times 10^6$/ml or 30% forward progression were included, while from the ICSI programme all patients with an ejaculated semen sample were included. The sperm samples were prepared mainly by a conventional swim-up procedure or, for sperm samples with very low concentration and/or forward progression, by centrifugation through Percoll gradients (45/70/80/90%).

Sperm concentration and percentage motility were determined before (Table I) and after preparation. For the morphological evaluation, a smear was made from 5–20 µl semen, and stained by the Sperm-Mac method (Oettlé, 1986). A ×1000 magnification was used, and the morphology of at least 100 sperm cells was determined for each sample. All slides were examined by the same technician, who was trained in the strict criteria method in a course led by Dr. Kruger, and who regularly participated in evaluating control slides. For comparison, the couples were divided into five major types of infertility within each morphology group: tubal factors, male factors, mixed male and female factors, unexplained infertility and other female factors (Table II).

The women were down-regulated with a gonadotrophin-releasing hormone analogue (Suprefact; Hoechst, Frankfurt am Main, Germany) for 3–5 weeks, and thereafter stimulated with daily i.m. injections of human menopausal gonadotrophin (Pergonal; Serono Laboratories, Geneva, Switzerland), or with daily s.c. injections of purified follicle stimulating hormone (Fertinorm; Serono), followed by human chorionic gonadotrophin (Profasi, 10 000 IU i.m.; Serono). Approximately 38 h later, the oocytes were aspirated using transvaginal ultrasound-guided retrieval.

In the IVF cycles, the oocytes were inseminated with a sperm concentration of 2×10^5/ml 4–5 h after oocyte aspiration and, in the ICSI cycles, the oocytes were denuded using hyaluronidase (80 IU/ml), whereafter one spermatozoon was deposited in the ooplasm of each oocyte. All oocytes were screened for pronuclei 18–20 h after insemination and, after a further 24 h, cleavage was analysed. One to three of the morphologically best embryos were transferred to the uterine cavity of the woman on day 2.

Initially, the correlation between the fertilization rate and the morphology was studied, and the cut-off values for our programme were determined, resulting in slightly different categories from those used by Kruger *et al.* (1988). The hereby established borderlines were then used for the rest of the study. Thereafter, the fertilization, pregnancy and abortion rates for each group were calculated.

Since many of the ICSI patients had impaired sperm parameters in addition to only morphology, these samples were further divided into two groups, one consisting of patients with morphology as the only 'abnormal' sperm parameter, and the other with one or several additional sperm factors being included (Table III). This division was made in order to determine whether it was morphology by itself or in combination with other parameters that had the greatest influence on fertilization, pregnancy and abortion rates.

Statistical analysis

The Mann–Whitney, Kruskall–Wallis and χ^2-tests were used for biometrical analysis of the data.

Results

Conventional IVF

As can be seen in Table IV and Figure 1, the fertilization rate for routine IVF correlated to the morphology. The cut-off point in our programme, where the fertilization rate decreased statistically between the 'normal' group and the 'good prognosis' group, was found to be 10% normal forms (68% fertilization $\geqslant$10% normal forms, 54% fertilization <10%, $P = 0.00002$). Below 5% normal forms, the overall fertilization rate was 35%, which was significantly lower than that for the good prognosis group ($P = 0.016$). However, fertilization was very patient-related in this poor prognosis group: 32% of the patients in this group had no or such poor fertilization that an embryo transfer could not be performed, while as many as 36% had a fertilization rate >50%. For those who did receive an embryo transfer, the pregnancy rate was as good in this group as in the others (Table IV). In addition, the abortion rate was lower in the poor prognosis group, resulting in a high rate of ongoing pregnancies.

ICSI

For the ICSI groups, no differences in fertilization or pregnancy rates were found (Table IV). Both the normal group and the poor prognosis group had spontaneous abortion rates lower or

Table II. The distribution of female age and infertility factors categorized according to the percentage of morphologically normal spermatozoa

	In-vitro fertilization % normal spermatozoa			Intracytoplasmic sperm injection % normal spermatozoa		
	<5%	5–9%	>10%	<5%	5–9%	>10%
Mean female age (years)	32.1	33.0	33.1	33.2	32.8	33.1
Tubal factor (%)	23	45	63	4	10	13
Male factor (%)	14	2	0.6	62	57	33
Mixed factors (%) (male and female)	23	16	9	23	20	26
Unexplained (%)	27	20	15	11	11	12
Other female factors (%)	13	17	12	–	2	16

Table III. The concentration and motility (mean and range) of the sperm samples used for ICSI divided into those with a teratozoospermic factor only (= one factor) and those with additional sperm factors (= 2–3 factors)

	$ICSI_1$ (=one factor) % normal spermatozoa		$ICSI_{2–3}$ (=2–3 factors) % normal spermatozoa	
	<5% (n = 11)	5–9% (n = 42)	<5% (n = 15)	5–9% (n = 28)
Concentration ($\times 10^6$/ml)	41 (20–90)	41 (20–100)	13 (2–30)	9 (2.5–40)
Motility (%)	38 (30–50)	45 (30–60)	40 (10–80)	47 (7–80)

Table IV. Comparison of the results after utilizing in-vitro fertilization or intracytoplasmic sperm injection

	In-vitro fertilization % normal spermatozoa			Intracytoplasmic sperm injection % normal spermatozoa		
	<5%	5–9%	>10%	<5%	5–9%	>10%
No. of aspirated cycles	22	88	355	26	70	61
Mean no. of aspirated oocytes	10.6	11.9	11.1	10.7	11.2	10.7
Mean ± SD fertilization rate (%)	35 ± 29	54 ± 33	68 ± 25	68 ± 19	68 ± 21	66 ± 17
Cycles with >50% fertilization (%)	36	65	82	96	87	89
Cycles with <10% fertilization (%)	27	17	3	0	1	0
No. of embryo transfers	15	71	337	26	66	53
Mean no. of embryos transferred	2.0	1.9	2.1	2.2	2.2	2.3
Embryo transfer rate (%)	68	80	95	100	94	87
Pregnancies/transfer (%)	33	35	32	31	29	23
Spontaneous abortion rate/pregnancy (%)	0	24	19	0 (1X)[a]	42	17
Ongoing pregnancies/embryo transfer (%)	33	27	26	27	17	19
Ongoing pregnancies/cycle (%)	23	21	25	27	16	16
Mean no. of 'good' embryos/aspiration	2.9	4.6	5.2	4.3	4.6	3.6
Patients with >2 'good' embryos available/embryo transfer (%)	67	72	65	54	67	58

[a]An extrauterine pregnancy was aborted.

similar to our overall rate, while the good prognosis group (5–9%) had a higher rate (42%).

When the ICSI cycles were split into two groups (Table V), one with poor sperm morphology as the only sperm parameter, it could be seen that a higher abortion rate for the sperm samples with 5–9% normal forms (Table IV) was not a feature of the group with only poor morphology but was found in the group with several sperm factors (31 versus 67%, Table V).

Discussion

When the Tygerberg group introduced their strict criteria, they set the borderlines between the three categories at 14 and 5% respectively. In our IVF programme, however, no statistically different fertilization rate could be seen between the groups with <14 and >14% normal forms. Instead, the cut-off was found at 10%, and therefore this value was used in the present study. The fertilization rate borderlines when using strict criteria are presumably variable for different IVF laboratories, and it would be wise not to adopt the Tygerberg system without first evaluating the results of the laboratory concerned. Our findings are in accordance with those of several other groups, who have also found their cut-off values to be <14% (e.g. Enginsu *et al.*, 1991; Ombelet *et al.*, 1994; Vawda *et al.*, 1996). It is also important to note that, even in the poor prognosis IVF group, almost 40% obtained a fertilization rate

of >50%, something which was also found in a recently published study by Vawda *et al.* (1996).

It is of course important to distinguish between fertilization *in vitro* and *in vivo*. Probably only a few spermatozoa reach the oocyte in the Fallopian tubes, and we know very little about the selection mechanisms in the reproductive tract of the woman. In a study by Freundl *et al.* (1988), it was found that most types of abnormal spermatozoa were unable to pass through the cervical mucus. Check *et al.* (1992) could not find any correlation between sperm morphology and pregnancies *in vivo* and, in studies from intrauterine inseminations, the same observation was made by Matorras *et al.* (1995), while Francavilla *et al.* (1990) found a statistically lower pregnancy rate in the presence of teratozoospermia. One reason for these discrepancies might be the fact that the first two groups used the Tygerberg strict criteria for evaluation, while Francavilla *et al.* used the WHO criteria.

In the present study, no difference was found in fertilization rate between the three groups when ICSI was used (Table IV and Figure 1). This indicates that the main fertilization problem lies either with sperm penetration of the zona pellucida and/or the fusion of the spermatozoon with the oolemma. This may also vary according to the abnormality occurring in the particular sperm sample. Mashiach *et al.* (1992) studied the ultrastructural morphology of sperm samples used in IVF and concluded that only the morphology of the head and the acrosome differed between fertilizing and non-fertilizing samples. It has also been shown by Menkveld *et al.* (1994) that the morphology of the acrosome can be used as a sensitive additional parameter, especially in the poor prognosis group. The morphology of the tail did not seem to matter in IVF, although it may be quite important in the in-vivo situation. Gordts *et al.* (1993) found that the fertilization rate improved significantly in both groups with <14% normal forms when using subzonal sperm injection (SUZI, i.e. the spermatozoon is introduced only as far as under the zona pellucida), and attained the same values as the normal morphology group. This indicates that the binding to and passage through the zona pellucida could be the major obstacle for the abnormal spermatozoon. Similar findings were observed by Cohen *et al.* (1991a,b) when performing SUZI or partial zona dissection with a teratozoospermic sample. Previous studies showed that several groups (Oehninger *et al.*, 1988; Enginsu *et al.*, 1992, 1993; Grow *et al.*, 1994; Ombelet *et al.*, 1994) increased the fertilization rate by using a higher concentration of spermatozoa in the insemination medium, but the pregnancy rate was increased in only two of these studies (Enginsu *et al.*, 1991, 1993).

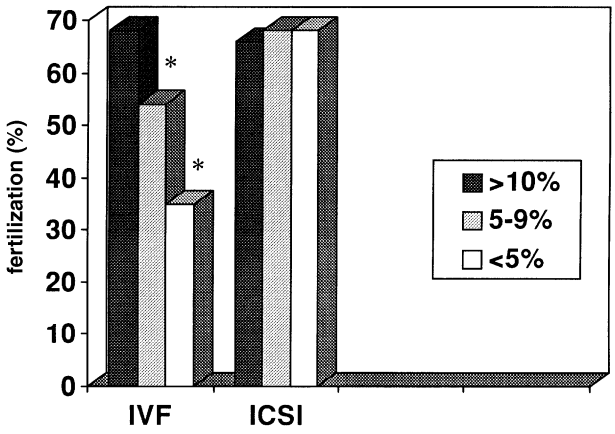

Figure 1. Fertilization according to sperm morphology (% normal forms) after utilizing in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI). Statistical analysis showed that the three groups undergoing conventional IVF were significantly different from each other (Kruskall–Wallis test, $P = 0.0007$), while the three groups undergoing ICSI were not ($P = 0.8601$).

It could be seen in the present study that a lower number of 'good' quality embryos (i.e. of grades 1 and 2) per aspirated patient was found in the poor prognosis group using routine IVF (Table IV). This was also shown by Cohen *et al.* (1991b) and Parinaud *et al.* (1993), who found that severe teratozoospermia resulted in embryos with more morphological abnormalities. However, the high pregnancy and low spontaneous abortion rates for this group in our study support the hypothesis that the morphological appearance of the spermatozoa does not correlate to inherent quality, and that once fertilization is attained with such spermatozoa, it does not result in embryos with a lower potential for development

Table V. Comparison of the intracytoplasmic sperm injection results between sperm samples which are divided into those with only one sperm factor (=teratozoospermic) and those with one or more additional factors

	Only teratozoospermia % normal spermatozoa		+ additional factors % normal spermatozoa	
	<5%	5–9%	<5%	5–9%
No. of cycles	11	42	15	28
Mean ± SD fertilization rate (%)	70 ± 21	68 ± 23	69 ± 12	70 ± 16
Cycles with >50% fertilization (%)	91	83	93	89
Cycles with <10% fertilization (%)	0	1	0	0
No. of embryo transfers	11	39	15	27
Embryo transfer rate (%)	100	93	100	96
Pregnancy rate/transfer (%)	36	33	27	22
Spontaneous abortion rate/pregnancy (%)	0	31	0 (1X)[a]	67
Ongoing pregnancies/embryo transfer (%)	36	23	20	7
Ongoing pregnancies/cycle (%)	36	21	20	7

[a]An extrauterine pregnancy was aborted.

and implantation. In addition, it is shown in Table IV that in this group 67% of the patients who obtained transfer had more than two high quality embryos available.

Moreover, it has not been possible to demonstrate any positive correlation between poor sperm morphology and an increased proportion of chromosomal abnormalities (Martin and Rademaker, 1988; Rosenbusch *et al.*, 1992). Concerning the rate of spontaneous abortion, the findings of this study indicate that poor sperm morphology, in itself, is not a factor that results in a higher spontaneous abortion rate, but rather that a combination of male factors may result in embryos with a lower potential for development.

In this study, the poor prognosis group (<5% normal forms) had the highest pregnancy rate per transfer and the lowest spontaneous abortion rate. This somewhat surprising finding was also noted by Yue *et al.* (1995), who proposed this to be due to the fact that all these men had a normal sperm count ($>20\times10^6$/ml), that the sperm samples were prepared on Percoll gradients (which could increase the morphology score) and also that a majority of the women did not have any infertility problems. That a higher number of 'healthy' women was found in the poor prognosis group was also noted in our study population (Table II).

Grow *et al.* (1994) found no statistically significant relationship between sperm parameters and pregnancy outcome, implantation rate or miscarriage rate in their overall material. However, when studying matched groups (maternal age, peak oestradiol, number of oocytes retrieved, number of embryos transferred and embryo grade at transfer), it was found that the implantation and ongoing pregnancy rates were significantly lower in the poor prognosis group than in the normal group, while no difference was seen in the miscarriage rate.

It is not possible, of course, to compare ICSI and IVF results completely, or to correlate ICSI to morphology, since ICSI involves the selection of one spermatozoon by the technician and many of the abnormalities are thereby probably bypassed. However, Nagy *et al.* (1995) showed that excellent results were obtained with ICSI even when utilizing a sample with 0% normal forms.

In conclusion, this study showed that poor sperm morphology (<5% normal forms) is a factor that, in approximately a quarter of the patients, results in impaired fertilization, and further that the problem can be solved by using ICSI. One way to find out which patients gain most from the ICSI technique is to divide the oocytes from each patient into two groups and perform both ICSI and IVF in the first cycle (so-called 'split cycles' or '50/50′ cycles). This is a method which is often used in our programme, giving us a chance to choose the optimal method for the next cycle.

It is also concluded that once fertilization is obtained in the poor prognosis group, either by using ICSI or conventional IVF, the chance of reaching a full-term pregnancy is good.

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