CLINICAL ASSISTED REPRODUCTION

Acrosome Index Is Not an Absolute Predictor of the Outcome Following Conventional In Vitro Fertilization and Intracytoplasmic Sperm Injection

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Purpose: To investigate if including evaluation of acrosome index (AI) in the semen analysis of teratozoospermic samples could help to predict for which patients intracytoplasmic sperm injection (ICSI) is necessary.

Methods: The fertilization rate, pregnancy rate, and percentage of good quality embryos were compared after performing conventional in vitro fertilization (IVF) and ICSI, respectively, using sibling oocytes. The role of AI was evaluated by dividing patients into two groups; Group A (AI < 7%) and Group B (AI ≥ 7%).

Results: A significant difference in fertilization rate was observed between Group A and B after conventional IVF. In Group A, the fertilization rate, embryo transfer rate, and percentage of good quality embryos were higher after ICSI than after IVF. In Group B, the fertilization and pregnancy rates were numerically but not significantly higher after IVF compared to ICSI.

Conclusion: Evaluation of acrosome index will not accurately predict fertilization, although this study shows that a sperm sample with less than 5% normal forms and an AI greater than 7% may achieve a mean fertilization rate >70% after conventional IVF.

KEY WORDS: acrosome index; fertilization; ICSI; IVF; sibling oocytes; teratozoospermia.

INTRODUCTION

Ever since the intracytoplasmic sperm injection (ICSI) method was introduced in assisted reproduction in the early 1990s, it has been of interest to find methods to predict which patients really benefit from this treatment, so as not to overuse the more invasive and potentially more risky ICSI technique. In 1986, the “strict criteria” for evaluating sperm morphology was introduced (1) and it was found that poor fertilization in conventional in vitro fertilization (IVF) occurred to a higher degree if the percentage of normal spermatozoa in the ejaculate was below 5% (poor prognosis group). Many groups have since reported that sperm morphology evaluated according to strict criteria is a good predictor for the fertilization rate in vitro (2–5). However, when ICSI is performed there does not seem to exist any relationship between sperm morphology and fertilization and/or pregnancy rates (6–10). In some of these studies, it was shown that a large group of patients obtained good fertilization rates after conventional IVF despite poor sperm morphology. In a previous study from our group (9), it was found that although the overall fertilization rate when using conventional IVF was significantly lower in the poor prognosis group compared to the good prognosis group (35 vs. 68%), a fertilization rate >50% was obtained with conventional IVF in as much as 36% of the cycles in the severely teratozoospermic group. Since ICSI is a more expensive and time-consuming technique, it would thus be very valuable to be able to distinguish this “high fertilization” subgroup from

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those patients where it is necessary to perform ICSI to obtain an acceptable fertilization rate.

It has been proposed (11,12) that the presence of morphologically normal acrosomes is of more importance for normal sperm–zona pellucida interaction than the overall sperm morphology alone, and that the morphology of the acrosome could thus be used as an additional parameter for predicting fertilization potential in conventional IVF. In previous studies (13,14), it was also shown that sperm morphology and a normal intact acrosome were significantly correlated to fertilization rate in vitro. The proportion of spermatozoa with intact acrosomes in the insemination medium was also significantly correlated to the fertilization rate in vitro, but only in the poor morphology group (<30% normal forms according to the WHO criteria).

The aim of this study was to investigate whether including evaluation of acrosome index (AI) in the semen analysis for the poor prognosis group could help to predict for which patients ICSI treatment is necessary to obtain an acceptable fertilization rate. Comparisons between IVF and ICSI treatment concerning fertilization rate, pregnancy rate, and percentage of good quality embryos were also performed.

MATERIAL AND METHODS

This prospective study performed from 1997 to 1999 consisted of 81 consecutive couples undergoing a split IVF/ICSI cycle at the Unit for Reproductive Medicine at Sahlgrenska University Hospital, Göteborg, Sweden. “Split cycles” or “50/50 cycles” are regularly performed at our unit if the percentage sperm morphology is below 5% and if at least one million progressive motile spermatozoa are obtained after preparation of the sperm sample by the swim-up procedure. This means that the oocytes from each patient are divided into two groups (if at least 8–10 oocytes are obtained) and both ICSI and IVF are performed in the first cycle. Sperm morphology, teratozoospermia index (TZI), and AI were analysed and possible correlation to the fertilization rate after conventional IVF and ICSI, respectively, was studied.

In Vitro Fertilization Protocol

The women were downregulated with the GnRH agonist Buserelin (Suprefact[®] or Suprecur[®] Hoechst, Frankfurt am Main, Germany) for 3–5 weeks, start-

ing on Cycle Day 21 or 1, and then stimulated with daily s.c. injections of recombinant FSH (Gonal-F[®], Serono Laboratories, Geneva, Switzerland, or Puregon[®], N.V. Organon, 5340 BH Oss, The Netherlands), followed by hCG (Profasi, 10,000 IU i.m., Serono). Oocyte retrieval was performed 36–38 h later by transvaginal ultrasound-guided pick up. Oocytes were collected alternately for IVF and ICSI. For conventional IVF, oocytes were inseminated with a sperm concentration of 200,000/mL 4–5 h after oocyte retrieval, and in the ICSI cycles oocytes were denuded using Hyase-10 (80 IU/mL) (Scandinavian IVF Science, Göteborg, Sweden) before ICSI was performed. All oocytes were screened for fertilization 18–20 h after insemination and the appearance of two pronuclei was considered normal fertilization. After a further 24 h of culture, embryo development was recorded. Embryos were scored as Grades 1–4, where Grade 1 embryos have even-sized blastomeres, no fragmentation, and a smooth cytoplasm without vacuoles. Grade 2 is defined as embryos with even or uneven-sized blastomeres and/or less than 20% fragmentation, and/or slightly nonhomogeneous cytoplasm. Grade 3 and 4 embryos have 20–50% or >50% fragmentation, respectively, and/or granulated cytoplasm with or without vacuoles. Transferable embryos were defined as Grade 1 or 2 embryos with at least 4 blastomeres on Day 2 or at least 6 blastomeres on Day 3. The best embryos were selected for transfer independently of the method used for the insemination (IVF or ICSI), although mixed transfers (i.e. one embryo from each IVF and ICSI treatment) were avoided as far as possible. Embryo transfer (ET) was performed if at least two embryos had reached the 4-cell stage. Otherwise culture was continued another 24 h before ET was performed. All embryos were cultured in IVFTM-50 medium (Scandinavian IVF Science, Göteborg, Sweden). In this study, a maximum of two embryos were transferred and all normally developed not-transferred embryos were cryopreserved. Fertilization and pregnancy rates were calculated as well as the percentage of good quality embryos (number of embryos good enough for ET or freezing divided by the number number of fertilized oocytes).

Semen Analysis

Ejaculates were collected after 3–5 days' sexual abstinence and were left to liquefy for 20–30 min. Semen analysis was performed according to the World Health Organization guidelines (15) except for the morphology, which will be described separately.

Sperm preparation was performed using a direct swim-up method where 0.5–1.0 mL of the ejaculate was added to the bottom of a conical 15 mL Falcon tube containing 1.5 mL of IVF medium. After incubation for 45–60 min at 37°C and 5% CO₂, 1.0 mL of the supernatant was collected and washed once with IVF medium at 200 × *g* for 5 min. The pellet was resuspended in IVF medium before evaluation of concentration and motility was performed. Samples were diluted to a concentration of 200,000/mL before insemination/microinjection.

Morphological Evaluation

For the morphological evaluation, thin smears were prepared using 5–10 µL of the ejaculate. Slides were air dried before fixation in 95% ethanol and staining with a modified Papanicolaou technique as previously described (16). Sperm morphology was evaluated according to strict criteria (17) and the acrosome morphology was evaluated on the same slide as described previously (11). Acrosomes were considered normal if clearly visible, well defined and comprising 40–70% of the sperm head. No more than two vacuoles were allowed. In addition to normality according to strict Tygerberg criteria, slightly abnormal as well as slightly and moderately elongated spermatozoa with normal acrosomes were classified as normal. These evaluations were always performed by the same trained technician and 200 spermatozoa were evaluated on each slide under oil immersion at a magnification of 1000×. To evaluate intraobserver reproducibility of the AI, five smears were reevaluated a total of 10 times each. Coefficients of variation varied between 5.6 and 11.4% (5.6, 11.4, 9.4, 8.6, and 6.9%). Head, neck, and tail defects or combinations of these were recorded separately and TZI (15) was calculated. AI was calculated by dividing the number of spermato-

zoa with normal acrosomes by the total number of spermatozoa evaluated.

Statistical Analysis

Results were calculated and presented by descriptive statistics and Student's pairs test was used for comparisons of IVF and ICSI results for the whole study population. The Mann-Whitney U-test was used for the comparisons between the different AI groups. To obtain the optimal AI cutoff value sensitivity and specificity were calculated for the different AI values. For categorical data, a Chi-square test in a 2 × 2 table was used. A *p* value <0.05 was considered statistically significant.

RESULTS

To obtain an AI cutoff value for 50% fertilization after conventional IVF sensitivity and specificity were calculated for the different AI values. The optimal cut-off level was found at an AI of 7%, giving a sensitivity and specificity of 81 and 62%, respectively. The 81 patients were therefore divided into two groups according to AI; Group A <7% and Group B ≥7% normal acrosomes. As can be seen in Table I, there was no statistically significant difference in male age or any of the sperm parameters between the two groups.

When comparing results for the whole study group (Table II), no significant difference was found in the overall fertilization rate between IVF and ICSI treatment. The percentage of good quality embryos and the possible ET rate (i.e. the percentage of cycles where enough good quality embryos to perform a transfer were obtained) was higher after ICSI treatment than after conventional IVF treatment, while the pregnancy rate per transfer was significantly

Table I. Semen Characteristics; Mean ± SD (Range)

	All	Group A (AI ^a < 7%; 1.5–6.5)	Group B (AI ≥ 7%; 7–14.5)	<i>p</i> -value
No. of patients	81	42	39	
Male age	34.9 ± 5.3 (24–47)	35.9 ± 5.4 (26–47)	33.9 ± 5.1 (23–47)	NS
Volume (mL)	3.5 ± 1.5 (0.5–9.5)	3.3 ± 1.4 (0.5–5.6)	3.8 ± 1.7 (1–9.5)	NS
Sperm count (×10 ⁶ /mL)	46.2 ± 36.4 (8–180)	47.0 ± 39.0 (10–164)	45.4 ± 33.8 (8–180)	NS
Motility (% motile)	46.2 ± 8.4 (30–80)	45.0 ± 7.7 (30–60)	47.4 ± 6.5 (30–80)	NS
Total motile count (×10 ⁶)	68.9 ± 56.8 (9–239)	61.8 ± 53.6 (9–230)	76.6 ± 59.8 (14–239)	NS
Morphology (% normal)	2.7 ± 1.1 (0–4)	2.6 ± 1.1 (0–4)	2.9 ± 1.1 (0–4)	NS
TZI	1.60 ± 0.20 (1.11–2.11)	1.60 ± 0.20 (1.11–2.11)	1.59 ± 0.19 (1.27–2.09)	NS

Note. NS *p* > .05.

^a AI = Acrosome index.

Table II. Results From IVF and ICSI, Respectively, All Cycles (Sibling Oocytes)

	IVF	ICSI	<i>p</i> -value
Number of cycles	81	81	
Mean number of oocytes inseminated ($\pm$ SD)	7.0 ($\pm$ 2.3)	7.1 ($\pm$ 2.5)	NS
Fertilization rate (mean $\pm$ SD)	57.2 ($\pm$ 31.7)	60.2 ($\pm$ 20.8)	NS
Embryo transfer rate ^a (%)	34/81 (42.0)	46/81 (56.8)	NS
Possible embryo transfer rate (%)	48/81 (59.3)	70/81 (86.4)	<.001
Pregnancy rate/ET ^b (%)	16/28 (57.0)	10/40 (25.0)	.004
Ongoing pregnancy/ET (%)	15/28 (53.6)	10/40 (25.0)	.008
Good quality embryos % of fertilized (mean $\pm$ SD)	38.1 ($\pm$ 38.5)	50.8 ($\pm$ 31.3)	.01
No. of cycles with no oocytes fertilized (%)	11/81 (13.6)	1/81 (1.2)	.003
No. of cycles with no good quality embryos (%)	33/81 (40.7)	11/81 (13.6)	<.001

Note. All mixed transfers are included in both IVF and ICSI cycles.

^a Including 6 mixed embryo transfers.

^b Excluding one pregnancy from mixed embryo transfer.

higher after conventional IVF than after ICSI (57.0% compared to 25.0%). Complete fertilization failure occurred in 13.6% of IVF cycles, and in total, in 40.7% of IVF cycles no good quality embryos were obtained, compared to 1.2 and 13.6%, respectively, in ICSI cycles (Table II).

When dividing results into AI Groups A and B (Table III), the fertilization rate, possible ET rate, and percentage of good quality embryos in group A were higher after ICSI than after conventional IVF. In Group B, the fertilization rate and pregnancy rate were numerically, but not significantly, higher after IVF compared to ICSI. The percentage of good quality embryos was similar after IVF and ICSI treatment.

DISCUSSION

In conventional IVF, the sperm must be able to bind to and penetrate the zona pellucida (ZP) as well as complete the acrosome reaction before fusing with the oolemma. A defective acrosome may impair fertilization by preventing the acrosome reaction occurring and thus inhibit the penetration (18). When using the ICSI technique, several of these fundamental sperm-egg surface interactions, including the acrosome reaction, are being bypassed.

ICSI is, however, a more invasive, time-consuming and also a more expensive procedure than conventional IVF, and many groups deal with the problem of

Table III. IVF and ICSI Outcome for the Different Acrosome Index Groups, Respectively; Mean $\pm$ SD (Range)

	Group A (<i>n</i> = 42)			Group B (<i>n</i> = 39)		
	IVF	ICSI	<i>p</i> -value	IVF	ICSI	<i>p</i> -value
Female age	32.0 $\pm$ 4.1 ^a (24–40)			33.0 $\pm$ 4.6 ^a (22–39)		
Number of oocytes inseminated	7.2 $\pm$ 2.1 ^a (4–14)	7.5 $\pm$ 2.5 (4–13)	NS	6.7 $\pm$ 2.4 ^a (3–13)	6.8 $\pm$ 2.5 (4–14)	NS
Fertilization rate	43.5 $\pm$ 33.7 ^b (0–100)	65.8 $\pm$ 20.2 (17–100)	.002	71.9 $\pm$ 21.5 ^b (9–100)	63.5 $\pm$ 21.6 (0–100)	NS
Possible embryo transfer rate (%)	19/42 (45) ^c	36/42 (86)	<.001	29/39 (74) ^c	29/39 (74)	NS
Pregnancy rate/ET ^f (%)	8/12 (67) ^a	8/27 (30)	.03	8/21 (38) ^a	2/20 (10)	NS
Good quality embryos % of fertilized	31.2 $\pm$ 39.7 ^d (0–100)	51.2 $\pm$ 30.9 (0–100)	.01	45.6 $\pm$ 36.2 ^d (0–100)	50.3 $\pm$ 31.7 (0–100)	NS
No. of cycles with no oocytes fertilized (%)	11/42 (26) ^e	0	<.001	0 ^e	1/39 (3)	NS

Note. For Group A, AI < 7%; for Group B, AI $\geq$ 7%.

^a NS.

^b *p* $\leq$.001.

^c *p* = .008.

^d *p* = .04.

^e NS (*p* < .05)

^f One pregnancy from mixed transfer was excluded in group A, giving a value of <0.001.

how to choose which couples really need to be treated by this method. Sperm morphology, at least when using strict criteria, is considered to be one important predictor for the outcome of fertilization and pregnancy after conventional IVF (1,4,5,17,19). However, it has been shown that good fertilization as well as pregnancy rates can also be obtained in the poor prognosis group (9,20–22).

In a previous study (11), promising results were presented from a group of 33 unselected patients where sperm morphology and AI were shown to correlate to fertilization rate in vitro. Additional results from an ongoing study of 110 patients have also been presented (12), where it was found that when using a cutoff point of $\geq 9\%$ normal acrosomes for $\geq 50\%$ fertilization in vitro the AI had a better predictive value than the overall sperm morphology alone.

In this study, 5 out of 42 patients in Group B ($AI \geq 7\%$) had a fertilization rate less than 50% (81% sensitivity), while more than half (21/39) of the patients in Group A ($AI < 7\%$) had $\geq 50\%$ fertilization (62% specificity). The cutoff value of 9% with high values for sensitivity (84%) and specificity (91%) reported (12) could thus not be confirmed in this study. One reason for this may be interlaboratory differences in the analysis of the AI. Another reason might be that in our study *only* poor prognosis patients were included while in the study by Menkveld *et al.* (11), sperm morphology varied between 1 and 22% normal forms.

Concerning the use of ICSI instead of IVF, it was seen in this study using sibling oocytes, that altogether 68% (55/81) of the patients obtained a fertilization rate of at least 50% with conventional IVF, while in 11 cycles out of 81 no oocytes were fertilized after conventional IVF (Table II). After ICSI treatment, only one cycle with complete fertilization failure occurred. This is in agreement with the results in another study also using sibling oocytes (10), where fertilization was obtained in 90% of IVF cycles and in 100% of ICSI cycles in the teratozoospermia group. In this study, the “possible ET” rates for ICSI- and IVF-embryos respectively are presented (Tables II and III) as in most cases ET was performed with embryos only from one method while the remaining good quality embryos were frozen. In 24 of 81 cycles (30%), no ET would have been possible without applying the ICSI technique.

In the study by Pisarska *et al.* (10), it was found that ICSI increased the number of fertilized oocytes by 18% compared to IVF in the group with severe teratozoospermia ($< 5\%$ normal forms) as the only cause of

subfertility. This was not confirmed in this study where the overall fertilization rate was similar for ICSI and IVF (Table II). When comparing ICSI and IVF results in Group B ($AI \geq 7\%$) (Table III), we found that the fertilization rate and pregnancy rate/ET rate were actually higher after conventional IVF compared to ICSI, although not significantly.

It has been proposed that poor sperm morphology may influence not only the fertilization rate but also early embryo development and fragmentation (23,24). In this study, approximately 27% of “ET failures” in the IVF group and 12% in the ICSI group were due to other reasons than poor fertilization, such as a poor cleavage rate or poor embryo quality.

It has been shown (25) that insemination using high sperm concentrations in patients with severe teratozoospermia can increase fertilization rates after conventional IVF, but did not result in more good quality embryos or higher pregnancy rates. In another study (26), it was shown that by insemination of at least 25,000 acrosomally normal spermatozoa in patients with poor acrosomal morphology, fertilization and pregnancy rates reached similar levels as in couples with normal acrosome morphology.

In a previous study (13) it was shown that the number of spermatozoa with intact normal acrosomes in general was increased by the swim-up procedure but this varied among different patients. Thus, poor acrosome morphology in the ejaculate can be compensated for by a higher concentration of acrosomally normal spermatozoa in the insemination medium. This might be one reason why the fertilization rate is still high in some patients with a poor AI.

It is obvious that there is a lack of standardization of methods used and there is also a high inter- and intralaboratory variability in the evaluation of sperm morphology. The reliability of results is therefore limited. In the future, computerized sperm analysis (CASA) may have the potential to eliminate subjectivity of manual readings and thereby increase accuracy and reproducibility. It would also be valuable if a more differentiated evaluation was possible, e.g. analysis of acrosome defects.

In conclusion, this study demonstrates that the AI evaluation will not accurately predict the fertilization potential of a poor prognosis sperm sample. It does show, however, that a sperm sample with a morphology below 5% normal forms and an AI above 7% has a mean fertilization rate following conventional IVF of approximately 70%, compared to a mean fertilization rate of approximately 40% for an $AI < 7\%$.

Performing an AI analysis in men with severe teratozoospermia as the only sperm factor (constituting approximately 20% of patients attending our IVF unit) imposes an extra workload on the IVF laboratory. This might be compensated for by the reduced number of ICSI cycles needed. However, in our opinion, the performance of 50% ICSI in the first treatment cycle for the poor prognosis patients seems to be the optimal method at present. If computerised morphological assessment is more readily available in the future, the inter- and intralaboratory variability may be greatly reduced, making it easier to perform comparative studies. Evaluation of sperm morphology and AI in combination with number of total motile spermatozoa available after sperm preparation may also prove to have a better predictive value for the choice of IVF or ICSI treatment than the basic sperm parameters alone.

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REFERENCES

- 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;46:1118–1123
- Enginsu ME, Dumoulin JCM, Pieters MHEC, Bras M, Evers JLH, Geraedts JPM: Evaluation of human sperm morphology using strict criteria after Diff-Quik staining: Correlation of morphology with fertilization in vitro. *Hum Reprod* 1991;6:854–858
- Grow DR, Swanson RJ, Oehninger S, Kruger TF, Seltman HJ, Muasher SJ, Toner JP: Sperm morphology as diagnosed by strict criteria: Probing the impact of teratozoospermia on fertilization rate and pregnancy outcome in a large in vitro fertilization population. *Fertil Steril* 1994;62:559–567
- Ombelet W, Fourie FL, Vandeput H, Bosmans E, Cox A, Janssen M, Kruger T: Teratozoospermia and in-vitro fertilization: A randomized prospective study. *Hum Reprod* 1994;9:1479–1484
- Vawda AI, Gunby J, Younglai EV: Semen parameters as predictors of in-vitro fertilization: The importance of strict criteria sperm morphology. *Hum Reprod* 1996;11:1445–1450
- Küpker W, Al-Hasani S, Schulze W, Kühnel W, Schill T, Felberbaum R, Diedrich K: Morphology in intracytoplasmic sperm injection: Preliminary results. *J Assist Reprod Genet* 1995;12:620–626
- Nagy ZP, Liu J, Joris H, Verheyen G, Tournaye H, Camus M, Derde M-P, Devroey P, Van Steirteghem AC: The result of intracytoplasmic sperm injection is not related to any of the basic sperm parameters. *Hum Reprod* 1995;10:1123–1129
- Svalander P, Jakobsson A-H, Forsberg A-S, Bengtsson A-K, Wikland M: The outcome of intracytoplasmic sperm injection is unrelated to 'strict criteria' sperm morphology. *Hum reprod* 1996;11:1019–1022
- Lundin K, Söderlund B, Hamberger L: The relationship between sperm morphology and rates of fertilization, pregnancy and spontaneous abortion in an in-vitro fertilization/intracytoplasmic injection programme. *Hum Reprod* 1997;12:2676–2681
- Pisarska MD, Casson PR, Cisneros PL, Lamb DJ, Lipshultz LI, Buster JE, Carson SA: Fertilization after standard in vitro fertilization versus intracytoplasmic sperm injection in sub-fertile males using sibling oocytes. *Fertil Steril* 1999;71:627–632
- Menkveld R, Rhemrev JPT, Franken DR, Vermeiden J, Kruger TF: Acrosomal morphology as a novel criterion for male fertility diagnosis: Relation with acrosin activity, morphology (strict criteria), and fertilization in vitro. *Fertil Steril* 1996;65:637–644
- Menkveld R, Stander FSH, Kruger TF: Comparison between acrosome index and teratozoospermia index as additional criteria to sperm morphology in the prediction of expected in-vitro fertilization outcome. *Hum Reprod* 1998;13:104
- Liu DY, Baker HW: The proportion of human sperm with poor morphology but normal intact acrosomes detected with *Pisum sativum* agglutinin correlates with fertilization in vitro. *Fertil Steril* 1988;50:288–293
- Liu D, Baker H: Test of human sperm function and fertilization in vitro. *Fertil Steril* 1992;58:465–483
- WorldHealthOrganization: WHO Laboratory Manual for the Examination of Human Semen and Sperm-Cervical Mucus Interaction. Cambridge, Cambridge University Press, 1992
- Menkveld R, Oettlé EE, Kruger TF, Swanson RJ, Acosta AA, Oehninger S: Atlas of Human Sperm Morphology: Baltimore, MD, Williams & Wilkins, 1991
- Kruger TF, Swanson RJ, Acosta AA, Matta JF, Simmons KF, Oehninger S: Predictive value of abnormal sperm morphology in in vitro fertilization. *Fertil Steril* 1988;49:112–117
- Liu DY, Bourne H, Baker HW: Fertilization and pregnancy with acrosome intact sperm by intracytoplasmic sperm injection in patients with disordered zona pellucida-induced acrosome reaction. *Fertil Steril* 1995;64:116–121
- Kruger TF, Matta JF, Acosta AA, Veeck LL, Simmons K, Swanson RJ, Brugo S: A new method of evaluating sperm morphology with predictive value for human in vitro fertilization. *Urology* 1987;30:248–251
- Robinson JN, Lockwood GM, Dokras A, Egan DM, Nicholson SC, Ross C, Barlow H: Does isolated teratozoospermia affect performance in in-vitro fertilization and embryo transfer. *Hum Reprod* 1994;9:870–874
- Coetzee K, Kruger TF, Lombard CJ: Predictive value of normal sperm morphology: A structured literature review. *Hum Reprod Update* 1998;4:73–82
- Höst E, Lindenberg S, Ernst E, Christensen F: Sperm morphology and IVF: Embryo quality in relation to sperm morphology following the WHO and Kruger's strict criteria. *Acta Obstet Gynecol Scand* 1999;78:526–529
- Ron-el R, Nachum H, Herman A, Golan A, Caspi E, Soffer Y: Delayed fertilization and poor embryonic development associated with impaired semen quality. *Fertil Steril* 1991;55:338–344

24. Parinaud J, Mieusset R, Vieitez G, Labal B, Richoilley G: Influence of sperm parameters on embryo quality. *Fertil Steril* 1993;60:888–892
25. Oehninger S, Kruger TF, Simon T, Jones D, Mayer J, Lanzendorf S, Toner JP, Muasher SJ: A comparative analysis of embryo implantation potential in patients with severe teratozoospermia undergoing in-vitro fertilization with a high insemination concentration or intracytoplasmic sperm injection. *Hum Reprod* 1996;11:1086–1089
26. Benoff S, Cooper GW, Paine T, Hurley IR, Napolitano B, Jacob A, Scholl GM, Hershlag A: Numerical dose-compensated in vitro fertilization inseminations yield high fertilization and pregnancy rates. *Fertil Steril* 1999;71:1019–1028